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INFLUENCE OF TEMPERATURE AND OTHER FACTORS ON THE MORPHOLOGY OF THE WHEAT SEEDLING [1]

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INTRODUCTION

The morphology of the wheat seedling (*Triticum vulgare* Vill.[2]) is affected by both soil temperature and light during the early stages of growth. Two of the more prominent and important seedling characters determined during early growth are the length of the coleoptile and the position or depth of the crown in the soil. The coleoptile, though its life is short, is of prime importance to normal germination. It is also the organ through which organisms causing some of the more serious plant diseases enter the seedling. The location of the crown in relation to the soil surface can readily influence tillering, crown-root development, heaving, and winter injury. Another seedling character affected by environment is the seminal root system.

The present study, conducted in the greenhouses at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., shows the influence of four constant soil temperatures on the seedling development of a spring and a winter wheat sown at two depths. In addition, some observations on certain factors influencing the seminal root frequency have been made.

REVIEW OF LITERATURE

The influence of depth of seeding on the coleoptile length of wheat was observed by Percival (*9*).[3] He also has described the two root systems and the factors influencing their formation and extent.

Dickson (*2*) noted the effect of soil temperatures on both coleoptile length and elongation of the subcrown internode of two varieties of wheat.

Kossowitsch (*5*) found that plants grown in shaded areas had higher crowns than those grown in full sunlight. He also showed in plant-house experiments that high temperatures gave a similar result. In addition, he gives field data showing that winter-killed plants had much shallower crowns than the surviving plants.

Tavčar (*12*) states that the variety least damaged by cold has the vegetative point of its principal stalk the most deeply buried.

Gladkii and Lykhvar (*4*) believe that soil moisture (about 18 percent) appears to be the main factor in the development of the under-

[1] Received for publication Oct. 5, 1935; issued May 1936.

[2] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L., but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writers give preference to that name.

[3] Reference is made by number (italic) to Literature Cited, p. 568.

node or coleoptile tiller. From 10 to 50 percent of the heads can come from this node.

Friedberg (*3*) found varietal differences in the rate of coleoptile tillering. He believes the coleoptile tiller can be a factor in recovery from winter injury.

MATERIAL AND METHODS

Well-developed kernels of Hard Federation (C. I.[4] 4733) and Turkey (C. I. 1558), both from Moccasin, Mont., were used. These varieties differ greatly in growth habit. Hard Federation is an early spring wheat with white kernels, introduced from Australia by the United States Department of Agriculture in 1915, and Turkey is a midseason, hardy, hard red winter wheat probably introduced into Kansas in 1873 by Russian Mennonites (*1*).

The seeds were sown in galvanized-iron cans 8 inches in diameter and 9½ inches deep, filled 7½ inches with a uniform loam. The desired moisture content was maintained by triweekly weighings and additions of water. Soil-surface evaporation was reduced by a coarse granular cork mulch.

For each variety there were 16 cans at temperatures of 12°, 16°, and 24° C. and 20 cans at 20° C. These were maintained constantly by thermostatic control. The mechanism of the tanks (controlling the temperatures) is described in detail by Leukel (*6*). Twelve seeds were sown to a can, and later the plants were thinned to four seedlings.

Two seedings, one made on December 5, 1928, and another on February 6, 1929, were studied. The most important difference between the two seedings, other than date with consequent light effects, was the depth of seeding. In the earlier sowing the seeds were 18 mm and in the later they were 45 mm below the top of the cork mulch.

The principal measurements made were length of coleoptile, length of subcrown internode, number of coleoptile tillers, number of tillers, and number of crown roots. The tillers and crown roots were counted when the plants were 8 weeks old in the first seeding and approximately 6 weeks old in the second.

EXPERIMENTAL DATA

GERMINATION

Germination of both Hard Federation and Turkey was most rapid at 24° C. in both seedings. At each lower temperature the germination period increased. As shown in table 1, no significant differences in the rate of germination between Hard Federation and Turkey occurred at any temperature.

EFFECT OF TEMPERATURE AND DEPTH OF SEEDING ON LENGTH OF COLEOPTILE

Soil temperature exerted a decided influence on the length of the coleoptile of the plants sown 18 mm deep (table 1). The average length of the coleoptile of both varieties grown at 20° C. was approximately 10 percent less than at 24° and from 25 to 28 percent less than at 16°. There were no significant differences in coleoptile length

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

between the plants grown at 16° and at 12°. When the seed was sown 45 mm deep, temperature differences had little effect on coleoptile length, as the greatest difference due to temperature in either variety was less than 6 percent.

TABLE 1.—*Effect of temperature and depth of seeding on plant characters of Hard Federation and Turkey wheats grown in controlled-soil-temperature tanks at the Arlington Experiment Farm, Rosslyn, Va.*

SEED SOWN 18 MM DEEP

Variety and soil temperature (° C.)	Planting to emergence	Coleoptile length	Subcrown-internode length	Planting to tillering	Tillers per plant	Plants with coleoptile tillers	Crown roots per plant
Hard Federation:	*Hours*	*Mm*[1]	*Mm*[1]	*Days*	*Number*[1]	*Percent*	*Number*[1]
24	92	54.9±0.57	31.7±0.51	33	2.9±0.06	0	4.2±0.20
20	104	49.3± .57	26.2± .38	28	3.3± .07	0	6.3± .21
16	178	41.4± .45	20.3± .33	28	3.8± .06	2	6.9± .15
12	211	42.3± .46	12.3± .32	33	3.1± .06	4	6.3± .15
Turkey:							
24	92	45.9± .47	22.3± .42	23	8.5± .28	0	6.9± .20
20	104	40.9± .40	19.8± .30	21	10.1± .26	6	7.5± .29
16	166	33.2± .31	13.5± .30	23	12.7± .19	56	5.4± .14
12	211	32.5± .24	3.9± .21	24	10.3± .16	79	3.4± .10
SEED SOWN 45 MM DEEP							
Hard Federation:							
24	96	61.5±0.33	33.5±0.51	26	2.7±0.07	2	5.6±0.11
20	122	61.8± .27	33.0± .43	26	2.7± .05	0	5.8± .12
16	161	61.5± .28	27.4± .42	25	2.6± .05	0	3.7± .12
12	218	58.6± .25	26.2± .35	34	2.7± .05	3	2.8± .09
Turkey:							
24	96	53.0± .22	24.7± .62	19	4.8± .16	0	3.8± .18
20	120	54.3± .22	21.6± .45	19	5.0± .12	4	2.4± .08
16	154	53.3± .17	15.7± .42	20	6.5± .17	48	1.8± .09
12	216	51.4± .16	14.6± .41	26	5.6± .09	87	1.2± .08

[1] The number following the plus-or-minus sign represents the probable error.

The effect of depth of seeding, or lack of light, on the coleoptile length is shown by comparing the data from the first and second seedings (table 1). At 24° C. the coleoptile length of the shallow-sown Hard Federation was 11 percent less than that of the deeper sown; at 20° it was 20 percent less, and at 16° it was 33 percent less. Turkey behaved similarly.

Hard Federation had a longer coleoptile than Turkey at all four temperatures, at both seeding depths. The varietal contrast was greater in the shallow seeding and increased inversely with temperature, ranging from 16 percent to 23 percent shorter for Turkey. When the seed was sown 45 mm deep, the varietal contrast differed but slightly with temperature, Turkey being only 12 to 14 percent shorter.

EFFECT OF TEMPERATURE AND DEPTH OF SEEDING ON LENGTH OF SUBCROWN INTERNODE

The location of the crown is dependent on the elongation of the internode or internodes between the basal attachment of the coleoptile and the first node of the crown. Normally, the latter is the node of the first foliage leaf, although in some cases it may be that of the second, third, or even fourth (fig. 1). The region between the coleoptile base and the crown comprises the subcrown internode or

internodes. The critical period determining the crown location, or the elongation of the subcrown internode, occurs soon after germination. Twelve days after sowing, or approximately 6 days after emergence, both at 20° and 24° C., the lower node of the crown of Hard Federation could be identified with the naked eye and was approximately in its final position.

FIGURE 1.—Wheat seedling showing development of the main crown at the second node rather than at the first.

At the temperatures and seeding depths used in these experiments the plants crowned normally, i.e., the first crown node was that of the first foliage leaf. The subcrown region above the coleoptile attachment consisted, therefore, of a single internode, the elongation of which determined the position of the crown.

In both Hard Federation and Turkey, the length of the subcrown internode increased progressively from 12° to 24° C., at both seeding depths. Temperature effects on subcrown internode length, as on coleoptile length, were greatest in the shallow-sown plants. When seeds were sown 18 mm deep the subcrown internode of Hard Federation averaged 17 percent shorter at 20° than at 24°, and that of Turkey 11 percent shorter. The subcrown internode elongation of Hard Federation at 16° and at 12° averaged 36 and 61 percent shorter, respectively, than at 24°, whereas in Turkey the elongation at 16° and at 12° was 38 and 83 percent shorter, respectively.

Deep seeding increased the length of the subcrown internode of both varieties at all four temperatures (table 1), the differences being slight at 24° C. but increasing at the lower temperatures. At 12° the internode of Hard Federation was 53 percent shorter from the shallow

seeding than from the deep seeding and that of Turkey 73 percent shorter.

Hard Federation crowned higher than Turkey at the four temperatures, in both seeding depths. The varietal contrast was sharp in all comparisons but most marked at 12°, especially in the 18 mm seeding, at which the crown of Turkey was 68 percent lower than that of Hard Federation. At the same temperature, Turkey, when sown 45 mm deep, crowned 44 percent lower than Hard Federation. At the two highest temperatures the difference was least, but still there was a minimum difference of 26 percent.

Hard Federation, when sown 18 mm deep and grown at 24°, 20°, and 16° C., crowned above the soil line, and Turkey behaved similarly at 24° and 20°. When the seed was sown 45 mm deep, the crown

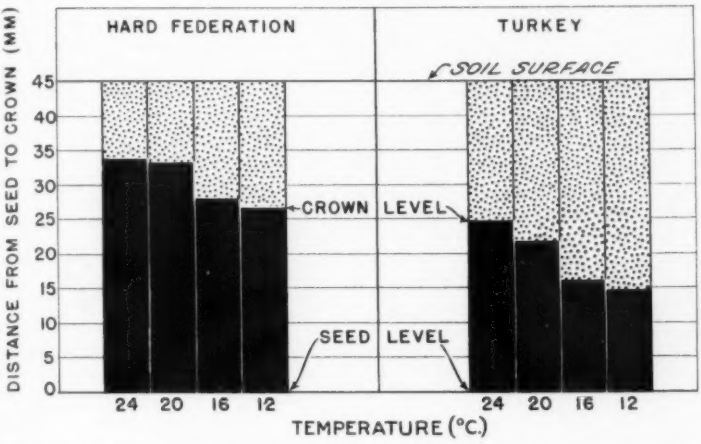

FIGURE 2.—Location of the crown with reference to the surface of the soil when the seed was sown 45 mm deep and grown at four constant temperatures

was well below the soil surface at all temperatures. The average position of the crown with reference to the soil surface at the deeper seeding is shown in figure 2.

EFFECT OF ENVIRONMENT AND VARIETY ON TILLERING

In the axil of each leaf, including the coleoptile and prophyll, there is a tiller bud. Inasmuch as each tiller has its prophyll, the extent of tillering is theoretically limited only by environment. However, it is generally accepted that varieties differ inherently in tillering proclivity.

In the writers' studies Turkey always tillered before Hard Federation. At the shallower seeding there was an average lapse of 23 days before the appearance of first tillers in Turkey and almost 31 days in Hard Federation. The corresponding intervals for the deeper seedings were 21 and 28 days. Hard Federation sown 18 mm deep and grown at 16° C. averaged 3.8 tillers per plant and Turkey averaged 12.7 tillers per plant. At all temperatures in both seedings (table 1), Turkey tillered more extensively than Hard Federation. In both

varieties, where differences were found, tillering was least at 24° and greatest at 16°.

The growth of the coleoptile axillary bud appears particularly sensitive to conditions during the early seedling development. When conditions are favorable the coleoptile axillary bud develops into the first tiller of the plant. Shortly afterward the tiller bud in the axil of the first seedling leaf develops into the second tiller, and the third tiller normally comes from the bud in the axil of the second leaf. The fourth tiller to appear may come either from the bud in the axil of the third leaf or from the prophyll axillary bud of the tiller in the

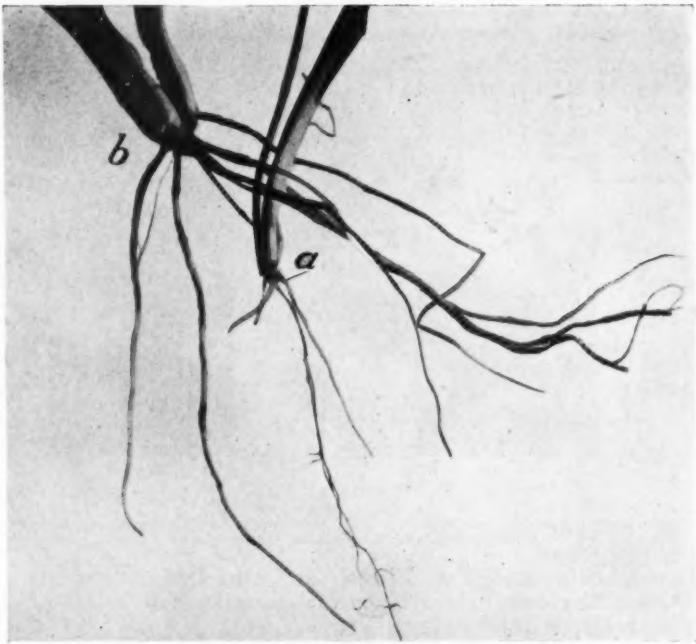

FIGURE 3.—Wheat plant with (*a*) crown developed from coleoptile tiller and (*b*) main crown.

axil of the first foliage leaf. In some varieties the coleoptile tiller fails to develop under most conditions. Theoretically, it would appear advantageous to have varieties tending to develop a tiller from the coleoptile node. This would produce a plant with two crowns, as shown in figure 3. Injury by low temperature, heaving, winds, or grazing by stock could kill or severely check the main crown and still leave the other capable of producing a crop. The coleoptile tiller forms what is in effect a second plant with its crown usually located deeper in the soil than the main crown. The significance of this second crown has been little investigated.

If the coleoptile tiller is to compete with the main crown tillers and develop significantly it must have an early start. Such a condition existed when the seeds of Turkey were germinated at 12° C., as the

first tiller recorded in all pots was from the coleoptile axillary bud The development of the coleoptile-node tiller is apparently determined by the availability of reserve food materials associated with the slow growth rate of the low-temperature environment.

As shown in table 1, Hard Federation rarely developed the coleoptile tiller at any of the four temperatures, the highest percentage being 4 at 12° C., with shallow seeding.

In Turkey, at 24° C., no coleoptile tillers were recorded; at 20° from 4 to 6 percent of the plants tillered from the coleoptile axil; while at 16° and 12° the percentage increased to approximately 50 and 80, respectively.

EFFECT OF ENVIRONMENT AND VARIETY ON EARLY FORMATION OF CROWN ROOTS

At the time counts were made more than 28 percent of the Hard Federation plants sown 18 mm deep at 24° C. had failed to develop functional crown roots. This was due to too high crowning. The formation of adventitious roots is very evidently closely associated with moisture. When plants grew so that the crown nodes came in contact with the moist soil, roots formed readily. Also, it was observed that crown roots formed even in the air at a sufficiently high degree of humidity. About 14 percent of the plants without crown roots had only the main stem, but the remaining 86 percent had one or more tillers. It is evident that formation of the first tillers may occur independently of crown-root functioning. The later degree of development of these tillers and of subsequent tillers, however, is undoubtedly closely associated with that of the adventitious crown roots.

The early development of tillers in excess of crown-root formation was most apparent in Turkey at 12° C. At the 18-mm depth of seeding the plants averaged 10.3 tillers but only 3.4 crown roots per plant. At the deeper seeding they averaged 5.6 tillers and only 1.2 crown roots. Hard Federation developed crown roots in excess of tillers under all conditions, but more at 12° in the 18-mm depth of seeding, averaging 6.3 crown roots and only 3.1 tillers (table 1).

EFFECT OF TEMPERATURE, DEPTH OF SEEDING, SIZE, AND PREMATURE HARVEST OF SEED ON NUMBER OF SEMINAL ROOTS

The seminal root system of wheat is not generally well understood, either in respect to number of roots or function. The information, however, is available. Wiggans (*15*) found that the seminal roots of common wheat varied from 1 to 6, and Locke and Clark (*7*) showed that the seminal root system may remain functional and carry the wheat plant through to maturity. McCall (*8*) found that there was a potentiality for as many as nine roots in the subcrown region which could be taken for seminal roots. However, the true seminal or seed roots are limited to a maximum of six: the primary, first and second pair of laterals, and a face root. At the coleoptile node, two, and occasionally three, adventitious roots may develop. These, however, should not be interpreted as seminal roots since their primordia are not present in the embryo. The development of the seminal roots is related to nutrition, and the full complement of six roots is rarely found.

Certain factors of possible influence in determining the number of seminal roots were investigated. These included temperature, depth of seeding, size of seed, and degree of seed maturity as determined by the interval between pollination and harvest.

Two seed sizes were used, the separation being made with a screen having 8 meshes per inch. The seed was germinated and grown at two temperatures—approximately 15° and 25° C.

The seedlings of Hard Federation differed but slightly in number of seminal roots when grown at 15° and at 25° C. The mean number of seminal roots at 15° was 3.5, and at 25° it was 3.4. The extreme numbers were 3 and 6. The number of seminal roots of Turkey was decidedly increased, however, by the higher soil temperature, as shown in figure 4. The mean number of seminal roots at 15° was 3.1, and at 25° it was 4.0. More than 90 percent of the seedlings at 15° had only 3 seminal roots, as compared with but 28 percent at 25°.

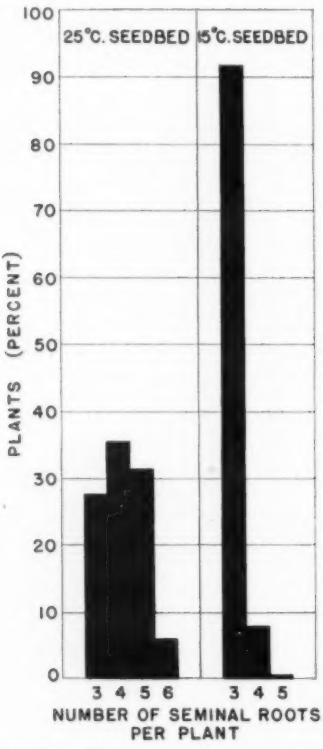

FIGURE 4.—Percentage of plants of Turkey wheat with different numbers of seminal roots when grown at soil temperatures of 25° and 15° C.

When the plants were grown at a soil temperature of 12° to 15° C., seeding 75 mm deep resulted in fewer seminal roots of both Hard Federation and Turkey than did seeding 20 mm deep. Turkey, however, was less affected by depth of seeding than was Hard Federation. More than three times as many plants with four seminal roots were found in the 20-mm seeding depth of Hard Federation as in the 75-mm depth (fig. 5).

The number of seminal roots was greater in both Hard Federation and Turkey plants grown from large seed. Turkey showed less effect, however, than Hard Federation, the mean number of seminal roots for the former from large seed being 3.7 as compared with 3.5 for the small seed. Plants from large seed of Hard Federation averaged 3.6 seminal roots and those from small seed 3.2 seminal roots. Figure 6 shows the effect of seed size on the seminal-root frequency of Hard Federation. More than 80 percent of the plants from small seed had but three seminal roots, while less than 50 percent of the plants from large seed had as few as three.

Robbins (*10*) found that the first pair of seminal rootlets appeared in the embryo about 4 weeks after pollination, and McCall (*8*) observed that wheat embryos varied in number of seminal-root primordia and that many did not show all six roots.

The number of seminal roots was determined on Purplestraw wheat seedlings from seed harvested 13, 20, and 30 days after flowering and cured in the shock. The results are shown in figure 7. The seed harvested 13 days after flowering produced about 50 percent of plants with three seminal roots and 15 percent with four seminal roots. The remainder were one- and two-root plants. The seed

harvested 20 days after flowering produced plants more than 60 percent of which had four seminal roots, and the seed harvested 30 days after flowering produced almost 70 percent of plants with five seminal roots. That these three seed lots differed decidedly in seminal-root production is evident, but whether these differences are due to the ages of embryos or to differences in food reserves is unknown.

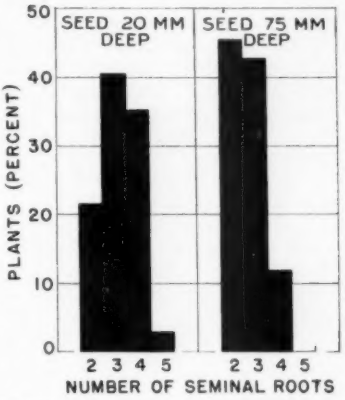

FIGURE 5.—Percentage of plants with different numbers of seminal roots from seed of Hard Federation wheat sown 20 mm and 75 mm deep.

COLEOPTILE-NODE ROOTS

The coleoptile-node roots, arising from just above the coleoptile node, are easily mistaken for seminal roots because in size and position they are so close to the true seminal roots. The number of the coleoptile-node roots appears to be related to food reserves. The relation of seed size, seeding depth, and age of embryo from pollination to the number of the coleoptile-node roots is shown in figure 8.

The number of coleoptile-node roots was much less from small seed and deep seeding, the effects of these conditions being about equal. Seed harvested 13 days after flowering produced plants with but few coleoptile roots as compared with seed harvested 20 and 30 days after flowering.

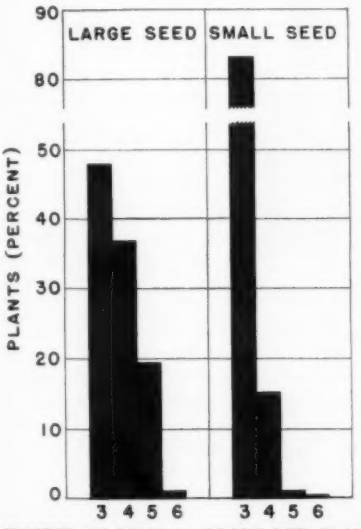

FIGURE 6.—Percentage of plants with different numbers of seminal roots from large and small seed of Hard Federation wheat.

DISCUSSION

Temperature and depth of seeding both have a marked influence on the morphology of the seedling wheat plant. When the seed was sown only 18 mm deep, much longer coleoptiles were produced at 20° and 24° than at 12° and 16° C. Deeper seeding, at 45 mm, however, was an even more potent factor than high temperature in increasing coleoptile length, both Hard Federation and Turkey having longer coleoptiles at 12° in the deeper seeding than at 24° in the shallow seeding. The effect of deep seeding on the length of the subcrown internode showed a similar trend, but temperature here apparently exerted more influence. A morphological study of either of these two characters without consideration of the seeding depth would be incomplete.

The effect of the position of the crown with relation to the soil surface on the later behavior of the plant has been little investigated. Locke and Clark (*7*) have shown that in dry regions, when the crown lies in dry soil, crown roots do not form. This was also noted by Webb and Stephens (*14*). A high-crowning variety, or environmental conditions that result in high crowning, would emphasize this reaction. Kossowitsch (*5*) cites field measurements showing that the plants that were winter-killed had much shallower crowns than those that survived. The wide differences between Hard

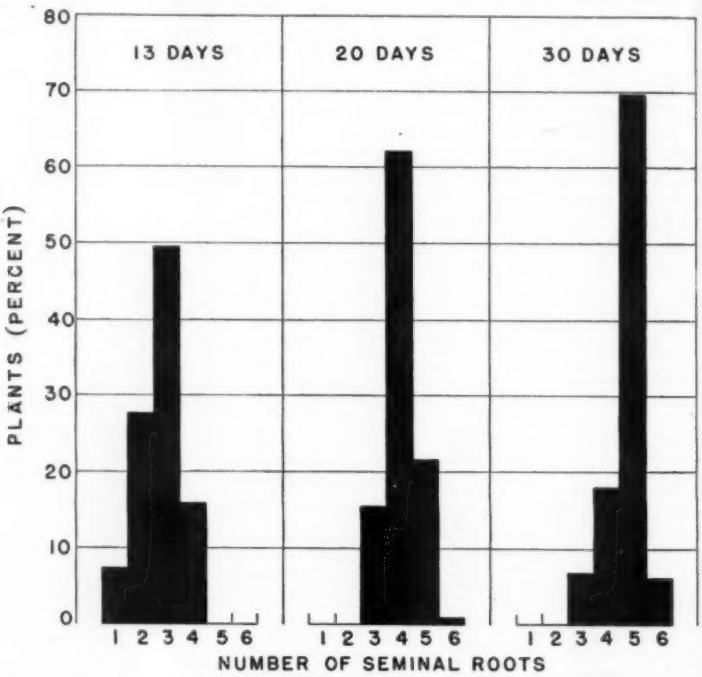

FIGURE 7.—Percentage of [illegible] urple straw seedlings with one to six seminal roots from seed harvested at three intervals after flowering.

Federation and Turkey in crowning habit, shown in these experiments and also by Webb and Stephens (*14*), indicate clearly that variety plays an important role.

The results here presented suggest that certain varietal differences in seedling wheat plants are largely determined by the degree of response to the temperature factor. The detrimental effects of too early sowing of winter wheat at a time when soil temperatures are still relatively high may well be caused, in part at least, by the effect of high temperature on crown position. The shallower crowns may be placed in drier soil, resulting in poor crown-root development. Later, cold injury may result from less protection or from poor root anchorage. These same factors would also be operative in periods of drought.

At favorable temperatures, Turkey possesses a decided tendency to develop a tiller and second crown from the coleoptile axillary bud. The crown resulting from this is usually located deeper in the soil than the main crown and, according to the observations of Kossowitsch (*5*), is less liable to winter injury. This second crown is therefore an additional safeguard. An analysis of varieties, both from the standpoint of temperature reactions in subcrown-internode elongation and in tendency to development of the coleoptile tiller, would seem useful as a basis for further improvement.

The slow development of the crown root system of Turkey may be one major reason why this variety often shows greater winter injury or killing in the eastern humid region than some of the varieties known to be less hardy at low temperatures. Heaving of plants from the ground is common, and in many years may be more serious than injury from low temperatures alone.

The importance of the seminal roots during the entire life cycle of the wheat plant has been well demonstrated by Simmonds and Sallans (*11*) and by Todaro (*13*). Whether it is important that the young seedling should have more than three seminal roots is entirely speculative in the light of present knowledge. On the basis of vigor, it could be assumed that a greater number is preferable. If so, this can be assured through seed quality, as measured by size and maturity.

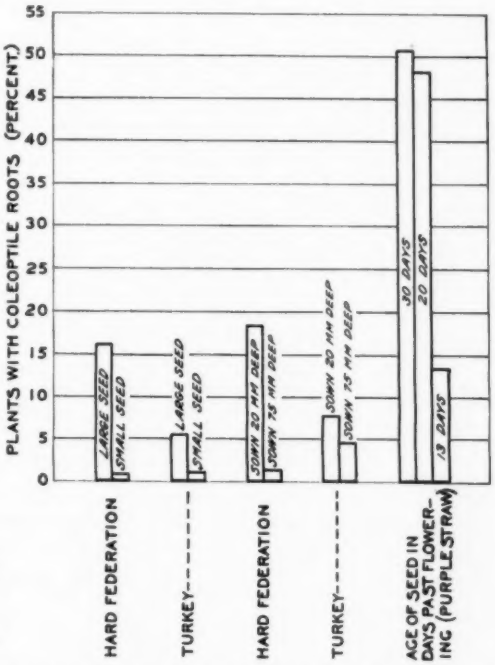

FIGURE 8.—Effect on number of coleoptile-node roots of the size of seed, depth of seeding, and age of seed from flowering.

SUMMARY

Temperatures of 24° and 20° C. increased the length of the coleoptiles of seedlings of Hard Federation and Turkey wheat as compared with temperatures of 16° and 12°. Deep seeding (45 mm) was more effective, however, than temperature in increasing the coleoptile length.

The length of the subcrown internode was also increased by both the high temperatures and the deeper seeding. In all comparisons, Hard Federation had a longer coleoptile and a longer subcrown internode than Turkey.

Coleoptile tillering seldom occurred at 24° or at 20° C., while at 16° approximately 50 percent of the Turkey plants tillered from the coleoptile node, and at 12° the percentage increased to more than 80. Hard Federation, however, tillered rarely from this node, as the highest percentage of plants with coleoptile tillers was but four.

The number of seminal roots of Turkey was increased by germinating the seed at 25° C. as compared with 15°. Large seed and well-matured seed also increased the number of seminal roots. With deep seeding (75 mm) the number of seminal roots was much less than with shallow seeding (20 mm deep).

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CROWN AND ROOT DEVELOPMENT IN WHEAT VARIETIES[1]

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INTRODUCTION

Spring grains grown under extremely dry soil conditions in certain parts of the Columbia River Basin often do not form crown roots but grow to maturity from seminal roots alone. Even fall-sown wheat occasionally may mature with few or no crown roots if it is sown shallow or if it emerges late in the fall and the following spring is dry. When the crown roots fail to develop, yields are reduced, and the plants break over at maturity just below the soil surface. Observations of the varieties grown at the Sherman Branch Experiment Station, Moro, Oreg., have indicated that there is an apparent varietal variation in elongation of the subcrown internode that determines the depth of the tillering nodes from which the crown roots develop. This suggests a possible correlation between the depth of the tillering nodes or crown and hardiness in wheat varieties, and the possibility that varieties may differ in the rate and ability to form crown roots under certain cultural and environmental conditions.

This paper presents the results from studies on some of the factors determining crown and root development, based on the examination of approximately 125,000 plants. The studies were started without the knowledge that somewhat similar investigations had been made by Tavčar (*14*)[2] and Friedberg (*4*) in Germany.

REVIEW OF LITERATURE

Taylor and McCall (*15*) have presented a review of the literature on effect of environmental factors on morphology of the wheat seedling. From experiments under controlled conditions they show that the subcrown internode and coleoptile elongate more when wheat is grown at higher temperatures than when it is grown at temperatures of 16° C. or lower. They also show that in deeper planting the crowns form deeper in the soil.

Weaver (*16*) found that crown-root development in wheat plants began with the appearance of tillers. Janssen (*7*) states that in winter wheat all new roots in the spring develop from the crown of the plant and not from old roots.

Worzella (*17*), in a recent investigation on root development, found that the nonhardy varieties of wheat had a tendency to root horizontally, whereas the hardy varieties rooted obliquely or straight downward.

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[2] Reference is made by number (italic) to Literature Cited, p. 583.

Many investigators have stated or implied that the seminal roots are only temporary. Others, however, notably Krassovsky (*8*) and Cravzov (*2*), contend that the temporary root system of cereals lives and functions until the death of the plant. Krassovsky found that in wheat salt absorption continued in the seminal roots throughout the vegetative period, reaching its maximum near the time of heading, when the main stem attains its maximum growth. She found a relation between the seminal roots and the main stem and between the crown roots and the tillers.

The common opinion among many investigators, including Carleton (*1*), Robbins (*13*), Locke and Clark (*9*), and Rabotnowa (*12*), was that the crown-root system is formed from nodes at a rather constant depth below the soil surface no matter how deep the seed is sown. Robbins states that the crown roots are formed at a depth of 1 inch below the surface, and Locke and Clark state that the crown roots arise usually just below the surface of the soil.

Govorov (*6*), Tavčar (*14*), and Friedberg (*4*) found that the depth of the tillering node varied with the variety and that the deeper situated tillering node was associated with winter hardiness. Dickson (*3*) and McKinney (*11*) found that the depth of the tillering node varied with different environmental factors, such as depth of seeding, amount of light, and temperature.

Friedberg (*4*) found that the tendency to produce tillers from the coleoptile node, which is a varietal character, allows of recovery after the growing point has been destroyed by frost; this explains why some varieties, although susceptible to frost, recover better than other varieties of equal susceptibility that seldom develop tillers from the coleoptile.

Gladkii and Lykhvar (*5*) found that varieties differed in ability to develop tillers from the coleoptile node and that the extent of development of tillers from this node increased with injury to the crown tillers.

EXPERIMENTAL METHODS

Varieties of winter and spring wheat were seeded at Moro, Oreg., on several dates during the year in order to get germination under different light and temperature conditions. After crown or root formation, which took place from 2 to 4 weeks after emergence, the tops of the plants were cut even with the ground with a sharp knife or scissors, and the crowns and subcrown internodes of the plants were dug. The distance from the seed and crown of each plant to the surface of the soil was recorded in millimeters. The difference is the length of the subcrown internode.

The term "seminal roots" is used in this paper to include those roots which form at the seed. According to McCall (*10*), the roots that arise from the coleoptile node are not true seminal roots. However, under field conditions it is difficult to distinguish them from the true seminal roots, primordia of which are present in the embryo. Crown roots are those that form at nodes above the coleoptile node. The internode above the coleoptile node, which elongates to determine the position of the crown, is the subcrown internode. The crown is the series of nodes with short internodes that forms usually below the surface of the soil. A tiller may be formed from each node in the crown, the number of tillers thus being determined by environment and variety.

EXPERIMENTAL DATA

VARIETAL VARIATION IN DEPTH OF CROWN

In 1931 a study was begun to determine the variation in crown depth of the winter wheat varieties in the plot trials at Moro., Oreg. These varieties were sown with a grain drill on October 15, 1930, and emerged November 6. At least 50 plants were examined in the spring of 1931 from each of three locations on each of three plots, or a total of 450 plants of each variety.

The study was continued in 1932, when 350 plants of each of the same varieties were examined. All varieties were sown with a grain drill on October 28, 1931, but owing to dry soil and cold weather, they did not emerge until early in March 1932.

The average depth of crown and length of the subcrown internode for the 25 varieties are given in table 1. The maximum and the minimum depths of crown found on any plants in 1931 are also given.

The data in table 1 show that the varieties ranked in about the same order for depth of crown in the 2 years. The varieties ranked in nearly inverse order for average length of subcrown internode, indicating that the depth of seeding averaged nearly the same for all varieties. The maximum and minimum crown depths found in plants of the same variety in 1931 show that considerable variability may occur.

TABLE 1.—*Depth of crown and length of subcrown internode of 25 varieties of winter wheat grown in the field at Moro, Oreg., in 1931 and 1932*

Variety	C. I. no.[1]	Depth of crown					Length of subcrown internode		
		1931 [2]			Average for 1932 [2]	2-year average	1931 [2]	1932 [3]	2-year average
		Maximum	Minimum	Average					
		Mm	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
Rio	10061	90	38	51	46	48.5	12	13	12.5
Golden	10043	80	33	54	39	46.5	8	21	14.5
Oro	8220	80	30	49	43	46.0	11	17	14.0
Turkey selection	11425	76	25	47	41	44.0	13	21	17.0
Kanred	5146	75	27	46	39	42.5	14	20	17.0
Kharkof	8249	79	30	46	38	42.0	15	22	18.5
Turkey (local)	4429	77	24	47	37	42.0	11	27	19.0
Imbler	10066	75	28	46	38	42.0	12	26	19.0
Triplet	5408	71	27	46	36	41.0	9	26	17.5
Turkey	1571	69	25	44	36	40.0	14	24	19.0
Turkey selection	11424	75	25	46	34	40.0	14	31	22.5
Kharkof	1442	71	25	41	36	38.5	16	24	20.0
Fortyfold selection 54	10064	70	23	41	36	38.5	21	20	20.5
Ridit	6703	78	18	40	36	38.0	21	25	23.0
Arco	8246	76	20	43	33	38.0	25	32	28.5
Rex	10065	60	20	40	34	37.0	20	32	26.0
Fortyfold selection 29	10062	65	28	41	33	37.0	20	31	25.5
P1068 × Preston	8244	70	21	36	36	36.0	23	24	23.5
Hybrid 128	4512	60	20	40	32	36.0	18	25	21.5
Arcadian × Hard Federation	11426	60	18	41	31	36.0	15	28	21.5
White Odessa	4655	65	17	37	32	34.5	20	28	24.0
Fortyfold × Federation	8247	65	20	38	31	34.5	28	26	27.0
Kanred × Marquis	11423	69	21	37	25	31.0	24	40	32.0
Fortyfold × Hard Federation	11422	60	18	36	26	31.0	20	43	31.5
Federation	4734	35	8	24	22	23.0	28	38	33.0

[1] C. I. refers to accession number of the Division of Cereal Crops and Diseases.
[2] Average of 450 plants.
[3] Average of 350 plants.

Varieties of the Crimean or Turkey group, and Golden, a selection from Goldcoin, had the deepest crowns. Oro, Rio, and Golden formed crowns more than 45 mm, or nearly 2 inches, below the soil surface. Federation, the only spring variety sown, formed crowns only about 23 mm, or less than 1 inch, below the soil surface (fig. 1).

Twenty-one winter wheat varieties were sown in nursery rows on October 17, 1933. Approximately 200 plants from each variety were examined in the spring of 1934. Table 2 gives their average depth of crown and length of subcrown internode. The average depth of crown ranged from 53 mm in Yogo and Turkey selection (C. I. 10016) to 28 mm in Federation. In this experiment, as in those made previously, the more winter-hardy wheats of the Turkey type formed their crowns deeper than did the less hardy, soft white wheats.

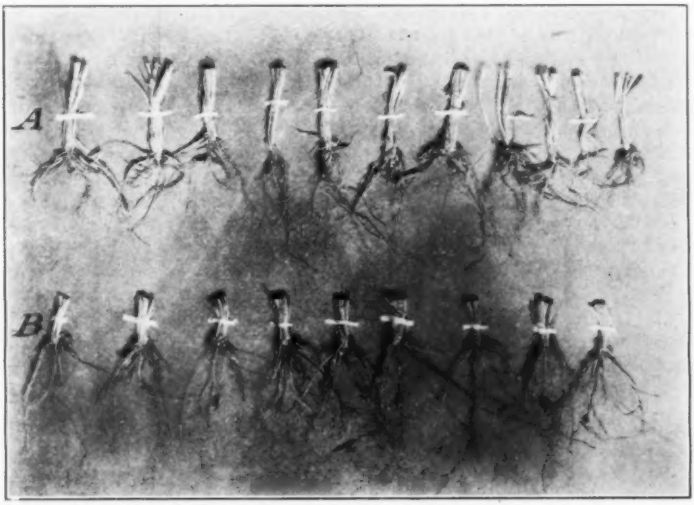

FIGURE 1.—Variation in crown depth of two wheat varieties (*A*, Rio; *B*, Federation) sown on the same date and at the same depth. The plants were cut at the soil surface.

Observations were made on depth of crown of 350 plants of each of 12 spring wheat varieties sown in the field at Moro on March 11, 1931. Similar results were obtained from the same varieties sown March 29, 1932, when 200 plants of each variety were examined. Table 3 gives the data for these spring wheat varieties. The crowns were formed nearer the soil surface than in the winter wheat varieties, and the differences in crown depth between varieties were smaller, the greatest being only 5.5 mm. The varieties, however, held about the same relation in the 2 years, indicating that spring wheat varieties do differ in the depth at which they crown.

TABLE 2.—*Depth of crown and length of subcrown internode of 21 varieties of winter wheat grown in nursery rows at Moro, Oreg., sown Oct. 17, 1933*

Variety	C. I. no.	Depth of crown [1]	Length of subcrown internode [1]	Variety	C. I. no.	Depth of crown [1]	Length of subcrown internode [1]
		Mm	*Mm*			*Mm*	*Mm*
Yogo	8033	53	9	Albit	8275	40	18
Turkey selection	10016	53	8	Triplet selection R4-29		39	23
Oro	8220	50	4	White Odessa	4655	39	21
Rio	10061	50	8	Hybrid 128	4512	38	19
Turkey selection	11376	47	9	Turkey × Florence N. No. 932		38	20
Minhardi	5149	47	12	Fortyfold × Federation	8247	36	23
Golden	10063	47	14	Hard Federation × Martin	11488	32	27
Kharkof	8249	46	13	Fortyfold × Hard Federation	11422	32	25
Kanred	4156	45	12	Federation	4734	28	31
Turkey selection	1532	44	15				
Ridit	6703	42	15				
Rex	10065	41	20				

[1] Average of 200 plants.

TABLE 3.—*Depth of crown and length of subcrown internode of 12 varieties of spring wheat grown in the field at Moro, Oreg., in 1931 and 1932*

Variety	C. I. no.	Depth of crown			Length of subcrown internode		
		1931 [1]	1932 [2]	2-year average	1931 [1]	1932 [2]	2-year average
		Mm	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
Marquis	4158	31	27	29.0	22	31	26.5
Pacific Bluestem	4067	30	25	27.5	24	33	28.5
Baart × Federation	8254	28	28	28.5	24	33	28.5
Baart	1697	28	25	26.5	25	33	29.0
Onas	6221	27	24	25.5	26	35	30.5
Federation	4734	27	24	25.5	30	36	33.0
Hard Federation selection 82	11421	27	23	25.0	30	34	32.0
Hard Federation selection 79	11420	27	23	25.0	30	42	36.0
Hard Federation	4733	26	23	24.5	31	37	34.0
Hard Federation selection 71	8256	27	22	24.5	31	35	33.0
Hard Federation 31	8255	27	22	24.5	31	37	34.0
White Federation	4981	25	22	23.5	30	39	34.5

[1] Average of 350 plants.
[2] Average of 200 plants.

VARIETAL VARIATION IN SUBCROWN PARTS

The internode above the coleoptile node usually is the only one to elongate below the crown in wheat plants. Under some conditions, however, one or more internodes above this one may elongate, and roots and a tiller may develop from each node.

Five wheat varieties were sown in small flats on May 14 and July 1, 1931, at Moro. After crowns had formed, 200 plants of each were examined to determine (1) the number of plants with two or more internodes immediately above the coleoptile node that had elongated and (2) the average length of these internodes. The data obtained are recorded in table 4. In these seedings some plants in all varieties elongated in two internodes, but only Golden produced many plants that elongated in three internodes.

TABLE 4.—*Percentage of plants with 1, 2, or 3 elongated internodes above the coleoptile node, and the average length of each internode, for several wheat varieties grown in Oregon under various conditions*

Item	C. I. no.	Depth of seed	First elongated internode		Second elongated internode		Third elongated internode	
			Plants	Length	Plants	Length	Plants	Length
Grown in field:								
Moro, 1931: [1]		*Mm*	*Pct.*	*Mm*	*Pct.*	*Mm*	*Pct.*	*Mm*
Federation	4734	64	100	46	28	14	4	6
Baart	1697	67	100	51	26	15	5	3
Hard Federation	4733	66	100	54	28	12	1	4
Golden	10063	61	100	39	29	11	13	23
Rio	10061	66	100	41	6	15	1	14
Moro, 1933: [2]								
Federation	4734	92	100	75	74	11	[3] 11	3
Hybrid 128	4512	99	100	62	79	16	1	1
Rio	10061	98	100	47	27	15	1	15
Oro	8220	98	100	48	35	15	0	.....
Corvallis, 1933: [2]								
Oro	8220	119	100	75	23	23	0	.....
Golden	10063	113	100	69	64	23	10	2
Grown in greenhouse:								
Corvallis, 1933: [2]								
Federation	4734	88	100	70	25	17	0	.....
Oro	8220	102	100	75	56	22	[3] 2	4

[1] Seeded May 14 and July 1; 200 plants of each variety were examined.
[2] About 100 plants of each variety were examined.
[3] 3 plants had 4 elongated internodes each.

Table 4 also gives data recorded in 1933 at Moro and Corvallis, Oreg., relative to elongation of second and third internodes above the coleoptile node.

The tendency of wheat plants to elongate in two or three internodes has been observed under various conditions, but the elongation occurs more frequently in plants seeded deep or under high temperatures.

In many varieties some plants were observed to form tillers from the axil of the coleoptile. This tendency seems to vary with varieties and with environmental conditions. Under certain conditions, as high as 50 percent of the plants in some varieties developed a tiller from the coleoptile node. It is possible for the coleoptile tiller to continue growth after the main crown is entirely removed. The development of the coleoptile tiller may be a distinct advantage to plants in recovering after the crown has been badly injured.

About 200 plants each of 21 winter wheat varieties grown in field plots at Moro were examined in the fall of 1934. The number of plants with coleoptile tillers developed was recorded, and the results are given in table 5. Plants with coleoptile tillers were more abundant in 1934 than in any previous year. The low temperatures and slow growth of the varieties apparently were favorable to the formation of coleoptile tillers, as was found by Taylor and McCall (*15*).

TABLE 5.—*Percentage of plants with coleoptile tillers in 21 varieties of winter wheat grown from seed sown in field plots Oct. 6, 1934, at Moro, Oreg.*

Variety	C. I. no.	Percentage of plants with coleoptile tillers [1]			Variety	C. I. no.	Percentage of plants with coleoptile tillers [1]		
		Nov. 23	Dec. 4	Average			Nov. 23	Dec. 4	Average
Turkey selection	11425	80	85	83	Hybrid 128	4512	52	60	56
Do	10016	68	81	75	Hybrid 128 × White Odessa	11607	34	75	55
Blackhull	6251	64	86	75	Rio	10061	51	58	55
Rex	10065	64	84	74	Turkey selection	11424	45	61	53
Marquis × Kanred	11423	60	77	69	Federation	4734	38	64	51
Turkey (local)	4429	53	75	64	Oro	8220	43	56	50
Ridit	6703	54	65	60	Cheyenne	8885	39	51	45
Kharkof	8249	53	65	59	Fortyfold × Hybrid 128	11700	43	47	45
Turkey	1571	55	63	59	Golden	10063	39	48	44
Kharkof	1442	45	69	57	Fortyfold × Federation	8247	28	48	38
Triplet	5408	48	65	57					

[1] Based on 100 plants examined in each variety on each date.

INFLUENCE OF DATE OF SEEDING ON DEPTH OF CROWN

Baart and Federation spring wheats were sown at 10- to 15-day intervals during the spring and summer of 1931. Each variety was seeded about 43 and 65 mm deep. The depths of the crowns for the various seedings are shown in table 6. The crowns formed much shallower during the summer, when temperatures were high, than during the spring or early fall, when lower temperatures prevailed.

Rio and Golden winter wheats and Hard Federation spring wheat were sown on November 2, 1930, and on several dates in 1931. As shown in table 6, each of the three varieties had comparatively shallow crowns except when sown on November 2, 1930, and March 14, 1931, when temperatures were low.

TABLE 6.—*Depth of crown of wheat varieties sown at various depths on several dates, and average air temperatures from seeding to 1 week after emergence, at Moro, Oreg., 1930–31*

Date of sowing	Average air temperature	Average depth of crown	Depth of crown of wheat varieties sown at indicated average depth						
			Winter varieties		Spring varieties				
						Baart		Federation	
			Rio (65 mm)	Golden (63 mm)	Hard Federation (64 mm)	43 mm	65 mm	42 mm	64 mm
1930									
Nov. 2	°F. 35	Mm 43	Mm 51	Mm 54	Mm 24	Mm	Mm	Mm	Mm
1931									
Mar. 14	44	[1] 30 and [2] 41	53	49	22	25	32	23	39
Apr. 6	49	30				30	32	23	34
May 1	58	22				20	23	22	23
May 14	61	20				20	20	18	20
July 1	68	20	31	22	8				
Aug. 2	68	15				12	16	15	18
Aug. 5	68	18	25	18	12				
Aug. 16	70	[1] 15 and [2] 19	21	21	14	13	14	15	17
Sept. 4	58	[1] 19 and [2] 22	23	26	18	18	18	17	21
Average			34	32	16	20	22	19	25

[1] Baart and Federation.
[2] Rio, Golden, and Hard Federation.

In 1933 four wheat varieties were sown at approximately 15-day intervals from July 26 to November 28. During this period daily maximum and minimum soil and air temperatures were recorded. The soil temperatures were obtained from a thermometer with the bulb placed about 2 inches below the soil surface. Shallow and deep seedings were made of each variety on each date. Table 7 shows the data obtained, including the depth of crown and seed for each variety under both shallow and deep sowing, and the average soil and air temperatures from date of seeding to 1 week after emergence.

TABLE 7.—*Depth of crown of four wheat varieties sown shallow and deep on several dates during the summer and fall of 1933, and the average mean air and soil temperatures from seeding to 1 week after emergence, at Moro, Oreg.*

Date of seeding	Average temperature		Average depth of crown	Depth of crown and seed, in millimeters, of indicated wheat variety sown shallow and deep															
				Federation				Hybrid 128				Rio				Oro			
				Shallow		Deep		Shallow		Deep		Shallow		Deep		Shallow		Deep	
	Air	Soil		Crown	Seed	Crown	Seed	Crown	Seed	Crown	Seed	Crown	Seed	Crown	Seed	Crown	Seed	Crown	Seed
	°F.	°F.	*Mm*																
July 26	71	76	21	12	48	17	92	17	46	22	87	20	39	27	88	22	39	29	89
Aug. 15	77	84	22	12	50	16	81	18	48	23	79	22	48	28	86	23	45	30	85
Aug. 31	60	66	26	18	37	21	85	21	40	25	82	27	42	31	83	29	45	35	87
Sept. 20	54	56	39	22	48	30	99	33	48	37	99	44	52	51	98	44	51	50	98
Nov. 14	42	39	41	26	53	31	105	35	56	41	101	46	56	52	87	43	52	50	78
Nov. 28	37	39	42	26	47	30	81	36	52	42	86	48	55	52	82	42	47	58	82
Average	----	----	------	19	47	24	91	27	48	32	89	35	49	40	87	34	47	42	87

As in previous years, the crowns of all varieties were much shallower when sown during the warm summer weather. The crowns were deeper, especially in the winter wheats, from the seedings made in the period from September 20 to November 28, when temperatures were lower. In the first few seedings, crown roots started about 3 to 4 weeks after seeding, whereas in the later seedings, a longer period was required before these roots started.

INFLUENCE OF DEPTH OF SEEDING ON DEPTH OF CROWN

Baart and Federation spring wheats were sown about 43 and 65 mm deep on April 12, 1931. The average air temperature from seeding to 1 week after emergence was 52° F. An examination of 250 plants of each variety from each depth of seeding showed that the crowns averaged 25 mm in the shallow seeding and 31 mm in the deep seeding.

Four varieties of wheat were sown at three depths in the field at Corvallis, Oreg., on March 21, 1933. The average depth of the crowns of 150 plants of each variety at each depth of seeding is given in table 8. The difference in crown depth between shallow-seeded and deep-seeded wheat is shown in figure 2.

TABLE 8.—*Depth of seed and crown in four wheat varieties sown at three depths in the field at Corvallis, Oreg., Mar. 21, 1933*

Depth of sowing	Average depth of crowns of 150 plants of—				
	Federation	Hybrid 128	Oro	Rio	Average
	Mm	*Mm*	*Mm*	*Mm*	*Mm*
Shallow (39 mm)	18	21	25	23	22
Medium (59 mm)	24	29	29	28	27
Deep (108 mm)	29	39	45	43	39
Average	24	30	33	31	

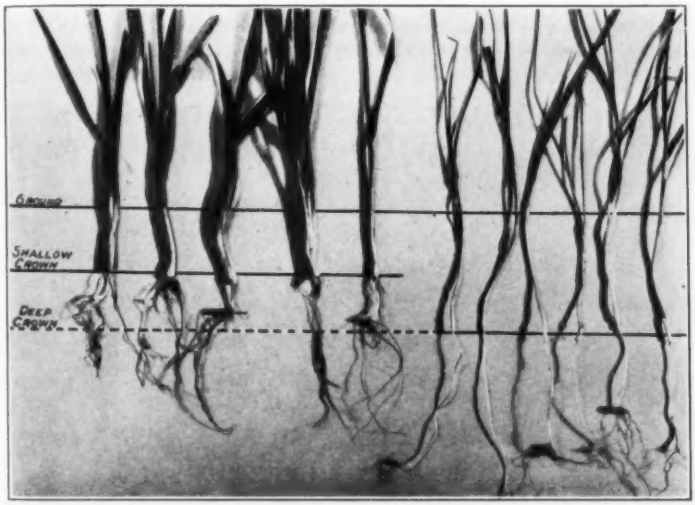

FIGURE 2.—Variation in crown depth between shallow (39 mm) seedings of Oro wheat, shown on the left, and deep (90 mm) seedings, shown on the right, which were sown on the same date. Because of earlier emergence, the shallow-seeded wheat (left) is further advanced in growth.

In the study on the effect of date of seeding on crown depth, made at Moro in 1934, each variety was seeded at two depths, as already shown in table 7. In all cases the deep-seeded plants produced crowns deeper on an average than those seeded shallow. The difference ranged from 3 to 16 mm, and the average depth of crown for all shallow-seeded plants in this study was 28.8 mm and for those seeded deep 34.5 mm.

Correlation coefficients between depth of seeding and depth of crown for Federation and Rio grown at Moro in 1932, are presented in table 9. A significant positive correlation was found between depth of seeding and depth of crown, especially with Rio, which crowns much deeper than Federation.

TABLE 9.—*Correlation coefficients between depth of seed and depth of crown for Federation and Rio, grown at Moro, Oreg., 1932*

Variety	Depth of sowing	Plants	Correlation coefficient between depth of seed and depth of crown
		Number	
Federation	Shallow	109	0.307±0.088
Do	Deep	105	.459± .077
Rio	Shallow	53	.671± .060
Do	Deep	228	.520± .049

Additional correlation coefficients were computed on part of the material grown at Corvallis, Oreg., in 1933. The varieties were separated into two groups, i. e., those normally shallow-crowned

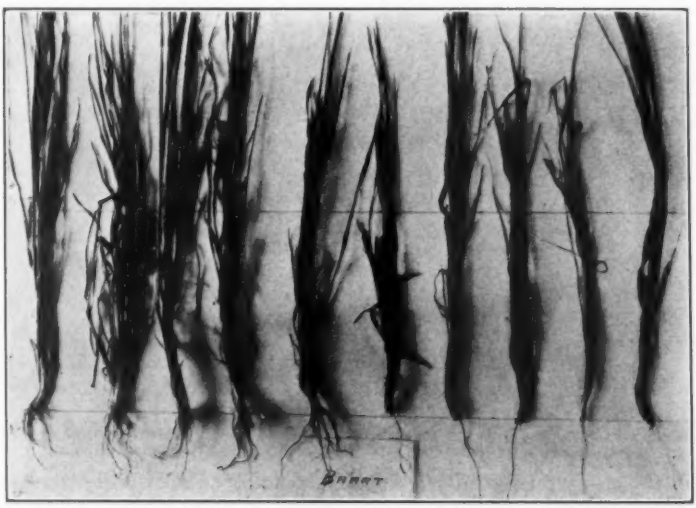

FIGURE 3.—Effect of rain during a dry spring upon the crown-root development of spring wheat (Baart) sown on March 9, 1934. Plants on the right were dug on April 21 and those at the left on April 26. A rain of 0.3 inch fell on April 24. Because of dry surface soil, there were no crown roots prior to the light rain, but 48 hours afterward well-developed crown roots had formed.

and those normally deep-crowned. The correlation coefficient between depth of seeding and depth of crown in 566 plants of the varieties that normally crown shallow was 0.753 ± 0.013, and in 505 plants of the varieties that normally crown deep, it was 0.765 ± 0.011. The data show that there was a close relationship between depth of seeding and the depth at which the crown was formed.

VARIETAL DIFFERENCES IN TIME OF CROWN-ROOT DEVELOPMENT

Because of dry surface soil, spring wheats often do not form crown roots at Moro, Oreg. (fig. 3). Under these dry conditions, a variety that begins growth of its crown roots sooner than another may have a distinct advantage.

In the spring of 1931, and again in 1932, 50 plants each of the 12 spring wheat varieties grown in the varietal experiment were examined on four dates, at 2- to 3-day intervals, to determine the percentage of plants on which crown roots were developing. The results, summarized in table 10, show that Federation and Onas, two high-yielding spring wheats at Moro, formed their crown roots somewhat earlier than did the other varieties in the trial.

TABLE 10.—*Percentage of plants of 12 spring wheat varieties having well-developed crown roots early in May, when varieties were sown Mar. 11, 1931, and Mar. 29, 1932, at Moro, Oreg.*

Variety	C. I. no.	Plants having well-developed crown roots on—			Variety	C. I. no.	Plants having well-developed crown roots on—		
		May 2 to 8, 1931[1]	May 3 to 11, 1932[1]	2-year average			May 2 to 8, 1931[1]	May 3 to 11, 1932[1]	2-year average
		Percent	*Percent*	*Percent*			*Percent*	*Percent*	*Percent*
Federation	4734	91	71	81	Baart × Federation	8254	57	44	51
Onas	6221	81	70	76	Baart	1697	58	42	50
Pacific Bluestem	4067	65	84	75	Hard Federation selection 79	11420	49	47	48
White Federation	4961	59	64	62	Hard Federation	4733	42	50	46
Marquis	4158	64	48	56	Hard Federation selection 71	8256	47	44	46
Hard Federation selection 82	11421	59	50	55					
Hard Federation 31	8255	54	52	53					

[1] 200 plants of each variety.

In the spring of 1934 a more extensive study of this nature was made on 10 of the 12 spring wheat varieties already studied. On March 9 the varieties were seeded in field plots with a grain drill. Counts were made on four dates of the number of plants with crown roots. These counts are recorded in table 11. This experiment was carried on under environmental conditions somewhat different from those in 1931 and 1932 in that the surface soil was so dry that crown-root formation was slow until after a rain of 0.3 inch on April 24. Up to that time, Pacific Bluestem and White Federation produced more plants with crown roots than the other varieties, as is shown in table 11 under date of April 21. After the rain, crown-root formation was very rapid, and in the final examination of 600 plants Pacific Bluestem and Federation ranked first and second, as shown under date of April 26. The percentage of increase between the third and fourth dates is given in the last column and shows that the most rapid increase in crown-root formation after the rain was in Federation.

TABLE 11.—*Percentage of plants of 10 spring wheat varieties having well-developed crown roots on Apr. 12, 16, 21, and 26, when varieties were sown on Mar. 9, 1934, at Moro, Oreg.*

Variety	C. I. no.	Plants having well-developed crown roots on—					Increase between Apr. 21 and Apr. 26 [4]
		Apr. 12 [1]	Apr. 16 [1]	Apr. 21 [2]	Apr. 26 [2]	Average	
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Pacific Bluestem	4067	27	47	52	90	54	73
Federation	4734	5	22	34	89	38	162
Baart × Federation	8254	8	29	38	88	41	132
White Federation	4981	13	42	50	86	48	72
Onas	6221	13	32	42	84	43	100
Hard Federation selection 71	8256	4	20	45	83	38	84
Baart	1697	4	20	43	82	37	91
Hard Federation	4733	11	35	43	82	43	91
Hard Federation 31	8255	5	28	32	81	37	153
Marquis	4158	6	18	35	71	33	103

[1] Approximately 250 plants of each variety.
[2] Approximately 600 plants of each variety.
[3] Rain occurred on Apr. 24.

In the fall of 1934, 200 plants each of the 21 winter wheat varieties grown in the varietal experiment at Moro, Oreg., were examined on two dates to determine the percentage of plants with crown roots developing. The results are given in table 12. It was found, as would be expected, that the rapid-growing, less hardy types of wheat formed their crown roots more rapidly than did the slow-growing Turkey types. This suggests a possible reason why in some localities certain soft winter wheat varieties that grow rapidly in their early stages and have a firmer root anchorage are better able to withstand injury from soil heaving than the slower growing, hardier Turkey types.

TABLE 12.—*Percentage of plants of 21 wheat varieties having well-developed crown roots on Nov. 23 and Dec. 4, when varieties were sown on Oct. 6, 1934, at Moro, Oreg.*

Variety	C. I. no.	Plants having well-developed crown roots on— [1]		Variety	C. I. no.	Plants having well-developed crown roots on— [1]	
		Nov. 23	Dec. 4			Nov. 23	Dec. 4
		Percent	*Percent*			*Percent*	*Percent*
Fortyfold × Federation	8247	79	87	Turkey selection	10016	2	14
Federation	4734	62	77	Marquis × Kanred	11423	2	7
Fortyfold × Hybrid 128	11700	35	69	Turkey selection	11425	0	4
Triplet	5408	41	64	Do	11424	2	3
Golden	10063	58	61	Turkey (local)	4429	0	3
Hybrid 128	4512	19	60	Kharkof	1442	0	2
Rex	10065	31	58	Do	8249	2	1
Blackhull	6251	11	48	Turkey	1571	1	1
Ridit	6703	16	38	Oro	8220	1	1
Hybrid 128 × White Odessa	11607	17	35	Rio	10061	0	1
				Cheyenne	8885	0	1

[1] Based on 200 plants of each variety on each date.

Further observations will be necessary before definite conclusions can be drawn as to the extent and importance of varietal variation in time of crown-root development, but the observations already

made indicate that the time after emergence at which these roots form and the rapidity of their growth are varietal characteristics.

DISCUSSION

The data presented in this paper and the results obtained by other investigators justify the conclusion that depth of crown is a varietal character in wheat. This character is greatly influenced, and varietal differences are sometimes obscured, by environmental conditions, especially soil temperature. No data were obtained on the influence of light on crown-root development, because no adequate light-control equipment was available. Light intensity may be a factor in influencing the position of the crown in a wheat plant, as indicated by Tavčar (*14*), Friedberg (*4*), and others, but from the results reported in this paper it is believed that soil temperature is a much more potent factor.

The depth of the crown and the character of the crown-root growth in the early stages of growth of a wheat variety may have considerable effect on the extent of winter injury. Wheat varieties cannot definitely be classified for hardiness, however, on crowning depth. Many factors are involved in winter hardiness, and depth of crown is only one. In two varieties otherwise equal in hardiness, the one with the deeper crown would be better able to withstand low temperatures. It has been observed that differences in winter killing in the same variety sown deep and shallow may sometimes be quite marked, if the variety is one that normally crowns deeply. Better survival may be obtained from deep seeding, because of the better protection to the crown or growing point. Of the wheat varieties studied at Moro, Oreg., for crown depth, all of the deeper crowned varieties are of the hardy Turkey wheats except Golden, which is only a moderately hardy selection from Goldcoin. All of the less hardy varieties, especially those with a spring habit of growth, had comparatively shallow crowns. Depth of crown varied from 48 mm for Rio to 23 mm for Federation in one experiment and from 53 mm for Yogo and Turkey selection (C. I. 10016) to 28 mm for Federation in another.

Ability to withstand low temperatures and winter injury from soil heaving may not be closely associated. A variety only moderately resistant to low temperatures, with an early and vigorous root growth, is better able to withstand injury from soil heaving than a variety very resistant to low temperatures but with less vigorous root growth in its early stages.

Small differences were found in the crown depth of the spring wheat varieties studied, and differences were observed in the time and rapidity of crown-root growth. The latter may be of importance in connection with the ability of varieties sown in the spring to establish an adequate root system before the surface soil becomes so dry as to inhibit root growth. Federation forms its crown roots more rapidly than do some other spring wheats under normal conditions; under dry conditions that inhibit crown-root development, Pacific Bluestem and White Federation appear to be able to make most rapid root growth. When sown in the fall, Fortyfold × Federation (C. I. 8247) and Federation form their crown roots much more rapidly than do the Turkey wheats.

The date of seeding affected the depth of crown of wheat varieties. Crowns were formed near the surface when seeds were sown in late spring or summer, when temperatures were high, and deep when seeds were sown in the fall or winter, when temperatures were low. When sown during cold weather, normally deep-crowned varieties may form crowns 45 to 50 mm below the surface, but when sown during warm weather they may have crowns only 20 to 25 mm deep.

The depth at which a wheat variety crowns may have an important bearing on the proper depth of seeding, a question of practical importance in the Columbia River Basin. The general recommendations in the past have been to seed winter wheat shallow, especially when the seeding is done late in the fall, when soil temperatures are low. Seeding too shallow, especially at early plantings, may result in more winter-killing. From the results of these studies it seems that seeding winter wheat much less than 38 mm deep would seldom be advisable. With favorable soil moisture and temperature conditions, winter wheat should be seeded from 51 to 64 mm deep, depending upon the variety. The best procedure would be to sow the seed at about the depth at which the crown would normally form, or slightly below. Deep seeding under normal conditions for winter wheat may produce crowns that will average 13 mm deeper than those produced by shallow seeding.

Depth of seeding influences the position of the crown, but the correlation is not high enough to warrant extremely deep seeding, even in spring wheat, in order to have deep crowns. Seeding spring wheat early and too deep will delay germination, emergence, and early root development. For late sowing, when soil temperatures are high, deeper seeding of spring wheat is advisable in order that the crowns may form as deep as possible.

SUMMARY

The depth below the surface of the soil at which the crown is formed in wheat plants is influenced by variety, environment (especially temperature), and depth of seeding.

Varieties differ greatly in the depth at which the crown is formed. Winter varieties form crowns deeper than spring varieties and, in general, hardy winter varieties form crowns deeper than nonhardy winter varieties.

Low temperatures between seeding and emergence cause the plants to crown deeper than do high temperatures.

Deeper seeding causes the crowns to be formed somewhat deeper but not in proportion to the difference in seeding depth.

Wheat varieties also differ in the time at which crown roots develop.

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CERTAIN INSECT VECTORS OF APLANOBACTER STEWARTI[1]

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INTRODUCTION

Bacterial wilt of corn (*Zea mays* L.) caused by *Aplanobacter stewarti* (E. F. Sm.) McC. was exceedingly destructive and more widely distributed during 1932 and 1933 than during any previous time in the history of the disease. Since 1897, when it was first described by Stewart, it has been studied by a number of investigators whose work has pointed more and more toward insects as a means of dissemination of the causal organism. Rand and Cash (*7*)[3] during 1920–23 found that bacterial wilt could be transmitted from diseased to healthy corn plants by two species of flea beetles, *Chaetocnema pulicaria* Melsh. and *C. denticulata* (Ill.), and by the spotted cucumber beetle, *Diabrotica duodecimpunctata* (Fab.). Ivanoff (*5*) reported transmission from diseased to healthy plants by the larval stage of the corn rootworm, *Diabrotica longicornis* (Say), as it attacked the roots of young seedling corn plants. He also reported that the bacteria of *A. stewarti* entered the corn plants through wounds made by white grubs, the larvae of *Phyllophaga* sp., feeding upon the roots in infested soil. A summary of this work, together with a brief review of the other literature on this disease, has recently appeared elsewhere (*1*).

The results of experiments by previous investigators on soil transmission of the causal organism indicate that transmission through the soil to uninjured roots of corn plants is exceedingly rare, if it ever occurs. Similar experiments by the writers have yielded only negative results. Therefore special attention has been given to insects as vectors of the organism, and the evidence of insect dissemination, obtained largely during 1934, is presented in this paper.

MATERIALS AND METHODS

In order to determine the importance of insects in the overwintering and dissemination of *Aplanobacter stewarti*, insects commonly found on corn during the growing season were collected from as many different localities and host plants as facilities for their study would permit. Insect vectors of this bacterial organism were determined

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[2] The writers are indebted to Nancy H. Wheeler and J. W. Scrivener, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for much valuable assistance with details of the experimental work reported in this paper; also to H. S. Barber of the same Bureau for his aid in identifying *Chaetocnema* spp., and to G. M. Smith, of the Bureau of Plant Industry, U. S. Department of Agriculture, for seed of inbred and hybrid corn used in the work.
[3] Reference is made by number (italic) to Literature Cited, p. 608.

by transferring various species which had been confined to infected corn plants in various types of cages to healthy corn plants in cages. Feeding habits and relative choice of host plants of the flea beetles (*Chaetocnema* spp.) were studied by confining single individuals of these species to the plants for a definite period by means of spring-clip cages [4] (fig. 1).

In order to determine which species of insects and what proportion of these contained *Aplanobacter stewarti* under field conditions, many species of insects collected on or near infected corn plants were disinfected externally in a 4-percent solution of sodium hydroxide for 7 minutes and then rinsed thoroughly in a 0.1-percent solution of hydrochloric acid before being crushed in sterile beef peptone broth for

FIGURE 1.—Cages used to confine single individuals of *Chaetocnema* spp. and other insects to leaves of corn and other plants. These cages are often attached to broadleaved plants by means of the spring only, hence the term, "spring clip cages."

plating. The percentage of individuals of any species of insect carrying *A. stewarti* was determined by plating from individual insects, previously disinfected externally and crushed in sterile beef broth in separate glass vials, 9 by 34 mm. Usually more than 100 individuals were tested in each experiment. A holder for the vials was made by boring holes of the proper diameter and depth in a 1- by 4- by 12-inch board, in which the vials were placed in an upright position and numbered serially.

[4] These cages were furnished by the Division of Truck Crop and Garden Insect Investigations of the Bureau of Entomology and Plant Quarantine.

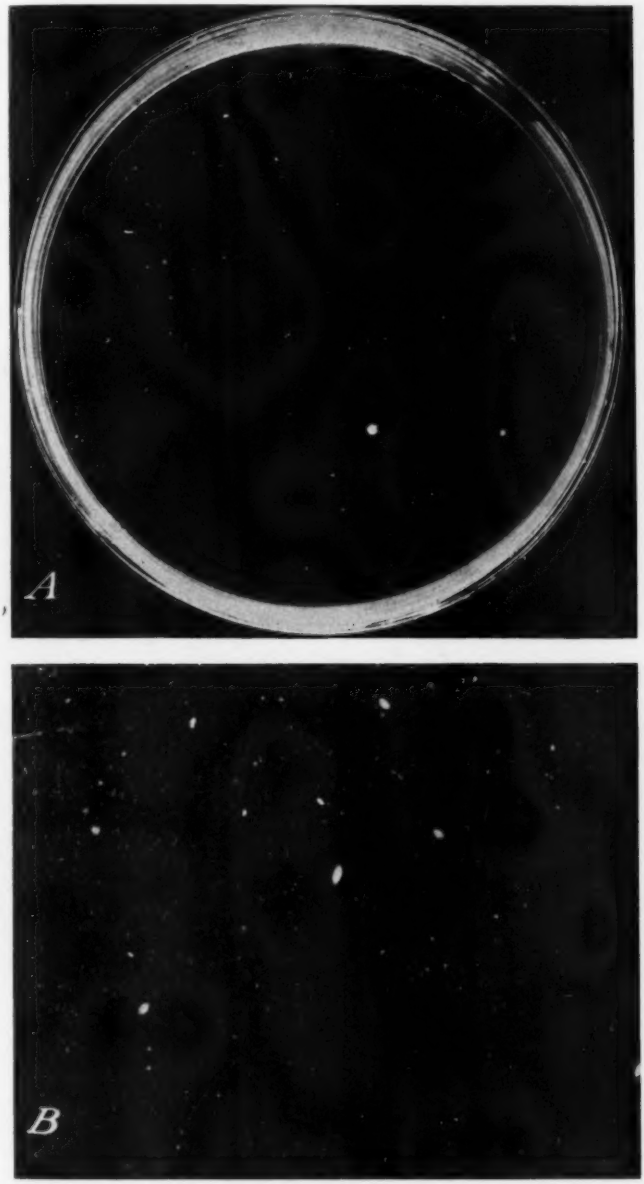

FIGURE 2.—*A*, Cultures of *Aplanobacter stewarti* isolated August 8, 1934, from *Chaetocnema pulicaria;* colonies too small to be seen on this plate. *B*, Small section of the plate shown in *A*, magnified 10 times. Photographed August 14, 1934.

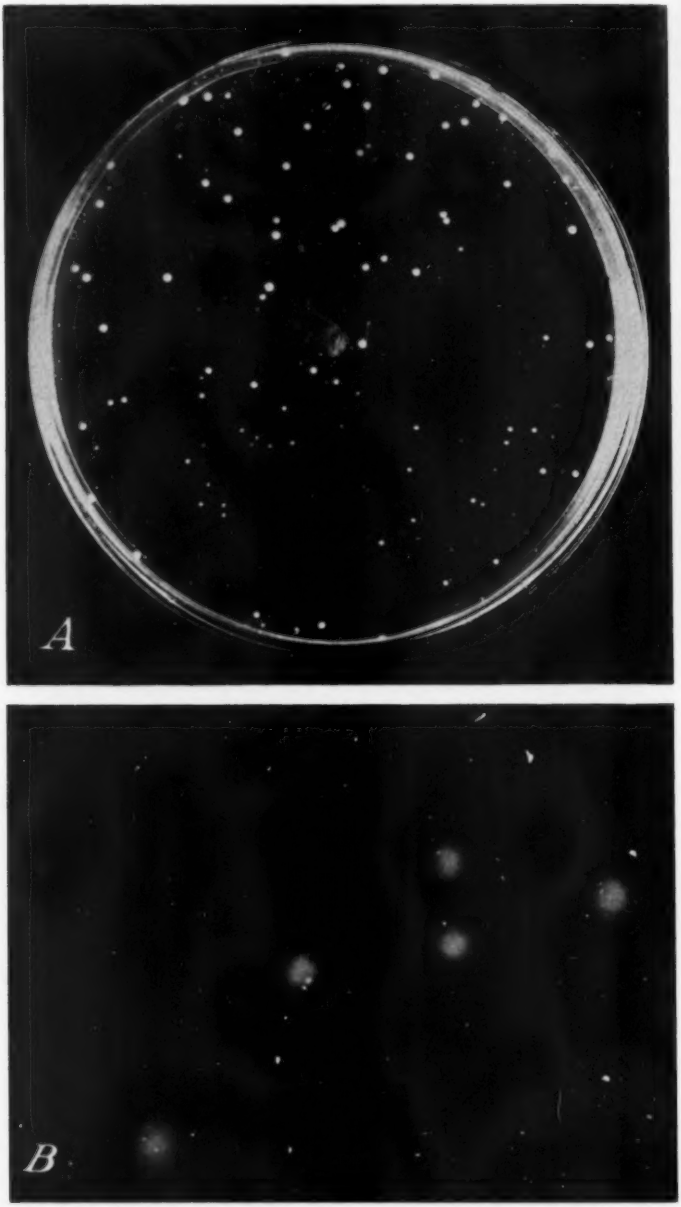

FIGURE 3.—*A*, Dilution plate (made from culture poured in figure 2, *A*) showing individual colonies of *Aplanobacter stewarti*. *B*, Small section of the dilution plate shown in *A*, magnified 10 times. Photographed August 14, 1934.

Isolations of *Aplanobacter stewarti* from the broth cultures containing the crushed insect material were made by pouring plates in Difco beef peptone agar pH 6.7. The broth suspension was added to the first tube of agar and a dilution made from this. In most of the isolations of *A. stewarti* heavy seedings and practically pure cultures of the organism were obtained. On the first plate (figs. 2, *A*, and 3, *A*) the colonies were too abundant to be distinguished by the naked eye. On the second or dilution plate (figs. 2, *B*, and 3, *B*) separate colonies 1 to 3 mm in diameter developed. Transfers from individual colonies of all isolations were tested for pathogenicity by the inoculation of susceptible corn plants under controlled conditions in the greenhouse (fig. 4).

FIGURE 4.—A portion of one bed in the greenhouse showing Golden Bantam sweet corn (Inbred 8482-3-1 from Glenn Smith) inoculated August 25, 1934, with isolations of *Aplanobacter stewarti* from *Chaetocnema pulicaria*. The large plants were inoculated but are free from the disease. Photographed September 11, 1934.

TESTS FOR TRANSMISSION BY INSECTS

At Arlington Experiment Farm, Rosslyn, Va., during 1933 and 1934, adults of *Chaetocnema pulicaria* (corn flea beetle), *C. denticulata* (toothed flea beetle), and *Diabrotica duodecimpunctata* (spotted cucumber beetle) were used successfully to transmit the bacterial wilt organism from diseased to healthy corn plants under controlled conditions. F. F. Dicke, of the Bureau of Entomology and Plant Quarantine, who was assigned to this project for a few months during 1933, obtained direct transmission of the disease from diseased to healthy corn plants by means of adults of the flea beetles *C. pulicaria* and *C. denticulata*. During January and February 1934 transmission from diseased to healthy plants was similarly effected by means of adults of the spotted cucumber beetle, *D. duodecimpunctata*.[5] The adult beetles were confined to corn plants, which were grown in 8-inch flowerpots, by means of cheesecloth-covered cages 2 by 2 feet by 2½ feet in height. A few attempts to transmit the disease from infected to healthy corn plants by means of the larvae of this beetle yielded

[5] These adults were kindly supplied by Charles E. Smith, in charge of a laboratory of the Bureau of Entomology and Plant Quarantine at Baton Rouge, La.

only negative results. The bacterial wilt organism was isolated in pure culture from the plants infected by these beetles.

Other insects were tested in a similar manner to some extent during the summers of 1933 and 1934, and only negative results were obtained. The adults of these species were confined to diseased corn plants for several days and were then transferred to healthy plants in various types of cages and under a wide variety of conditions. The list of species is as follows:

Chaetocnema confinis Crotch	Sweetpotato flea beetle (a. n. o.).[6]
Empoasca fabae (Harr.)	Potato leafhopper (a. n. o.).
Lygus pratensis (L.)	Tarnished plant bug (a. n. o.).
Nabis ferus (L.)	Nabid bug.
Systena taeniata blanda (Melsh.)	Pale-striped flea beetle (a. n. o.).
Chlorotettix viridius Van D.	Leafhopper.
Ceratomegilla fuscilabris (Muls.)	Spotted ladybird.
Thyanta custator (Fab.)	Pentatomidae (stinkbug family).
Phlepsius irroratus (Say)	Irrorate leafhopper.
Melanoplus differentialis (Thos.)	Differential grasshopper (a. n. o.).
Cicadula divisa (Uhl.)	Six-spotted leafhopper (a. n. o.).
Heliothrips femoralis Reut	Thrips.
Halticus citri (Ashm.)	Garden flea hopper (a. n. o.).

The species of insects which have given positive results in the transmission of the wilt organism from diseased to healthy corn plants seem to be unequally endowed with the ability to induce infection. In the light of information now available it seems that *Chaetocnema pulicaria*, *C. denticulata*, and *Diabrotica duodecimpunctata* transmit the disease with facility in the order named. Many more lesions developed from feeding injuries of *C. pulicaria* than from those of the other two beetles. *C. pulicaria* also occurred in much greater abundance on corn throughout the season at Arlington Experiment Farm than either of the other two species.

OVERWINTERING OF APLANOBACTER STEWARTI

During April 1934 the beetles *Chaetocnema pulicaria* and *C. denticulata*, which had hibernated as adults, were collected from orchard grass (*Dactylis glomerata* L.), rye (*Secale cereale* L.), and alfalfa (*Medicago sativa* L.) at Arlington Experiment Farm and tested (as described under Materials and Methods) for the overwintering of *Aplanobacter stewarti*. A brief report on these studies has already been published (*2*). Comparatively few *C. denticulata* were available for this purpose and no wilt organisms were obtained from this species. *C. pulicaria* was fairly abundant, and, at first, collections of 100 or more were crushed together in one lot of broth. After large numbers of colonies of *A. stewarti* had been secured in practically pure culture from four such collections of these insects, 175 *C. pulicaria* collected from several different species of host plants were crushed individually, each in about 10 cc of the broth, in order to determine approximately what percentage of these beetles harbored the organism over winter. These tests indicated that approximately 19 percent of the beetles contained the organism in abundance. In the case of 100 *C. pulicaria* collected from alfalfa on April 30, one isolation was made from the abdomens and another from the heads and thoraxes. Heavy seedings

[6] Americano nomina officinale, i. e., common name accepted by the American Association of Economic Entomologists.

of *A. stewarti* were obtained from both. On May 1, 1934, two isolations were made from each of six adults of *C. pulicaria* collected from orchard grass, one isolation from the abdomen and one from the head and thorax. A heavy seeding of *A. stewarti* was obtained from the abdomen of one of these six flea beetles and a lighter seeding from the head and thorax of the same beetle.

As a further proof of the overwintering of *Aplanobacter stewarti* within the corn flea beetle, healthy corn plants in the greenhouse were infested for several days with other adult beetles from the collections referred to. Typical symptoms of bacterial wilt developed in these plants (fig. 5), and *A. stewarti* was isolated from them in pure culture.

FIGURE 5.—Sweet-corn plant inoculated with *Aplanobacter stewarti* by confining overwintered adults of *Chaetocnema pulicaria* to it for a few days during April 1934.

INSECT VECTORS OF APLANOBACTER STEWARTI IN THE FIELD DURING THE GROWING SEASON OF CORN

Isolation of *Aplanobacter stewarti* from externally sterilized insects was continued throughout the summer and fall to determine what percentage of the *Chaetocnema* collected from different host plants and localities were infested and to learn whether other insects also were concerned in the transmission of this disease under favorable field conditions. The results of this work are summarized in table 1.

Most of the insects collected were taken from or near corn infected with the disease during the active growing season. This fact explains the small numbers used in the case of several of the species tested, as they were found only occasionally on corn. In the case of *Chaetocnema pulicaria* and *C. denticulata*, the figures in the table include all individuals of these species from field collections which were tested, and also those used in the studies on overwintering of the organism within the insects. In several instances collections of these species were made from field corn at some distance from any known source of the disease in order to compare the results obtained with those of collections from or near infected corn. In several cases single cultures

represented many insects, sometimes over 100, instead of only single individuals.

TABLE 1.—*Summary of insects collected in the field and cultured for Aplanobacter stewarti, 1934*

Scientific name	Common name	Total specimens	Localities	Species of hosts	Isolations made	Isolations tested by inoculations on corn	Isolations of *A. stewarti*
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
Chaetocnema pulicaria Melsh	Corn flea beetle (a. n. o.)	4,790	29	12	3,360	1,330	916
Chaetocnema denticulata (Ill.)	Toothed flea beetle	905	16	9	384	89	30
Chaetocnema confinis Crotch	Sweetpotato flea beetle (a. n. o.).	173	9	6	32	7	2
Diabrotica duodecimpunctata (Fab.)	Spotted cucumber beetle (a. n. o.).	178	3	5	20	7	0
Diabrotica larvae		3	1	1	1	0	0
Epitrix cucumeris Harr	Potato flea beetle (a. n. o.)	365	13	6	49	5	0
Epitrix parvula (Fab.)	Tobacco flea beetle (a. n. o.)	12	2	1	8	1	0
Carabid	Ground beetle	1	1	1	1	1	0
Dibolia borealis Chev	Leaf beetle	42	2	2	3	0	0
Disonycha xanthomelaena (Dalm.)	Spinach flea beetle	1	1	1	1	0	0
Disonycha glabrata (Fab.)	Flea beetle	2	1	1	1	1	1
Systena taeniata blanda (Melsh.)	Pale-striped flea beetle (a. n. o.).	2	1	1	1	0	0
Phyllotreta vittata (Fab.)	Striped flea beetle (a. n. o.)	6	1	1	3	0	0
Stilbus viduus Casey	Shining flower beetle	22	1	1	3	1	0
Stilbus apicalis (Melsh.)	do	7	1	1	2	1	1
Phalacrus politus Melsh	Smut beetle (a. n. o.)	19	1	1	3	2	0
Collops quadrimaculatus (Fab.)	Beetle	3	1	1	2	0	0
Epilachna corrupta Muls	Mexican bean beetle (a. n. o.)	1	1	1	1	0	0
Hypera punctata (Fab.)	Clover leaf weevil (a. n. o.)	3	1	1	1	0	0
Fulgorid	Lantern fly	3	1	1	1	0	0
Empoasca spp	Leafhopper	47	1	1	4	3	0
Eutettix seminudus (Say)	do	2	1	1	2	0	0
Deltocephalus flaricosta Stål	do	1	1	1	1	0	0
Euscelis bicolor (Van D.)	do	4	1	1	1	1	1
Agallia constricta Van D	do	11	1	1	2	0	0
Agallia sanguinolenta (Prov.)	Clover leafhopper	2	1	1	2	0	0
Cicadula divisa (Uhl.)	Six-spotted leafhopper (a. n. o.).	40	1	1	2	1	0
Polyamia inimica (Say)	Inimical leafhopper	1	1	1	1	1	0
Thamnotettix nigrifrons (Forbes)	Black-faced leafhopper	132	1	1	5	2	1
Draeculacephala mollipes (Say)	Tenderfoot leafhopper	5	1	1	1	0	0
Illinoia solanifolii (Ashm.)	Potato aphid (a. n. o.)	155	2	1	55	5	1
Anaphothrips obscurus (Müller)	Grass thrips (a. n. o.)	66	2	1	9	1	0
Aeolothrips fasciatus (L.) / *Anaphothrips obscurus* (Müller)		50	1	1	1	0	0
Orius insidiosus (Say)	Insidious flowerbug	220	1	1	5	2	0
Geocoris punctipes (Say)	Plant bug	3	1	1	2	0	0
Lygus pratensis (L.)	Tarnished plant bug (a. n. o.)	50	1	1	2	1	0
Adelphocoris rapidus (Say)	Plant bug	1	1	1	1	1	0
Nabis ferus (L.)	Nabid bug	1	1	1	1	1	0
Micrutalis calva (Say)	Tree hopper	1	1	1	1	0	0
Sminthurus sp	Springtail	8	1	1	2	0	0
Total (40 different species)		7,338			3,977	1,464	953

All of the insects tested, except the aphids and one lot of thrips, were sterilized externally before being crushed in the broth for plating. Samples of material from the collections of the various species used for isolations were referred to taxonomic specialists in the Bureau of Entomology and Plant Quarantine for identification.[7]

As indicated in the last column of table 1, isolations of *Aplanobacter stewarti* were obtained under these conditions from the following eight species: *Chaetocnema pulicaria*, *C. denticulata*, *C. confinis*,

[7] This assistance is hereby gratefully acknowledged.

Disonycha glabrata, *Stilbus apicalis*, *Euscelis bicolor*, *Thamnotettix nigrifrons*, and *Illinoia solanifolii*. From all of these, except the *Chaetocnema* spp., *A. stewarti* was obtained in only a single instance. However, only small numbers of each of these species were taken directly from infected corn. Additional work is therefore necessary to determine the importance of these species as vectors of this organism. The effective isolation of *A. stewarti* from *I. solanifolii* was obtained from a lot of 48 individuals collected from infected sweet corn at Burlington, N. J., on July 17.

It is possible that the positive results obtained with *Chaetocnema confinis* may be due to mistaken identification, because the external characters given for distinguishing this species from *C. pulicaria* are probably not entirely reliable, and the whole genus appears to be greatly in need of a taxonomic revision. It is also very difficult to identify specimens of these species in a living condition because of their extreme activity. In some cases these insects were placed on a small cube of ice under the binocular microscope in order to separate the species from each other in a living condition. This method was not always satisfactory because in hot weather a film of moisture soon appeared on the thoraxes of the specimens and prevented a clear examination of the punctation there.

TABLE 2.—*Percentage of Chaetocnema pulicaria infested with Aplanobacter stewarti at Arlington Experiment Farm, 1934*

Date collected	Host plant	Adults cultured	Proportion yielding wilt organism	Date collected	Host plant	Adults cultured	Proportion yielding wilt organism
		Number	*Percent*			*Number*	*Percent*
May 1	Orchard grass	18	11.1	Aug. 1	Sudan grass	100	19.0
14	Sweet corn	85	.0	8	do	66	12.1
16	Orchard grass	4	25.0	10	Sweet corn	90	50.0
16	Wheat	57	17.5	10	Johnson grass	10	20.0
16	Sweet corn	54	18.5	15	Alfalfa and grass	100	9.0
18	do	83	13.2	19	Sweet corn	100	36.0
June 18	Johnson grass	100	.0	28	do	100	47.0
20	Field corn	50	44.0	Sept. 5	do	100	58.0
20	Sweet corn	50	40.0	11	Sudan grass	100	6.0
July 9	do	100	75.0	19	Sweet corn	46	56.5
12	Bindweed	17	5.8	19	Sudan grass	47	2.1
31	Sweet corn	100	38.0				

As reported in table 1, 4,790 specimens of *Chaetocnema pulicaria* were collected from 12 different host plants and from 29 localities during 1934. Of the 3,360 isolations made from this species, 916 yielded colonies of *Aplanobacter stewarti*, and transfers from these colonies produced typical wilt symptoms on young corn plants in the greenhouse. The host plants from which these *C. pulicaria* were collected were sweet corn, field corn, black bindweed (*Polygonum convolvulus* L.), alfalfa, young wheat (*Triticum aestivum* L.), Sudan grass (*Sorghum vulgare sudanense* (Piper) Hitchc.), Johnson grass (*Sorghum halepense* (L.) Pers.), tomato (*Lycopersicon esculentum* Mill.), oats (*Avena sativa* L.), cowpea (*Vigna sinensis* (L.) Endl.), yellow bristlegrass (*Setaria lutescens* (Weigel) F. T. Hubb), sedge grass (*Cyperus* sp.), and weeds. The total of 908 adults of *C. pulicaria* collected from sweet corn at Arlington Experiment Farm during the

period May 14 to September 19 yielded an average of 40.3 percent infested with the wilt organism. Of the 669 adults of *C. pulicaria* collected from various other host plants (including field corn) during the same period at Arlington Experiment Farm, 12.1 percent were infested with *A. stewarti*. The results of tests with *C. pulicaria* collected at Arlington Experiment Farm during 1934 are presented in table 2.

The maximum proportion of *Chaetocnema pulicaria* from field collections found to contain *Aplanobacter stewarti* was 75 percent. These beetles were collected from infected sweet corn July 9.

Collections of *Chaetocnema pulicaria* from various localities have shown varying percentages of beetles which yielded the wilt organism when cultured in the usual manner. The results of these studies are given in table 3.

TABLE 3.—*Percentage of Chaetocnema pulicaria from various localities infested with Aplanobacter stewarti, 1934*

Date collected	Host plant	Locality	Adults tested	Proportion yielding wilt organism
			Number	*Percent*
May 5	Winter oats	Society Hill, S. C.	112	0.0
7	Field corn	Charleston, S. C.	145	.0
11	Oats	Norge, Va.	59	.0
June 6	do	Rising Sun, Md.	67	16.4
6	Field corn	do	50	6.0
6	Sweet corn	Princeton, N. J.	8	.0
6	Tomato	do	72	.0
10	Oats	Sussex, N. J.	3	.0
22	Field corn	Falls Church, Va.	50	28.0
22	Sweet corn	Annandale, Va.	50	16.0
27	do	Greenville, Pa.	16	25.0
27	Field corn	do	5	60.0
July 2	do	Falls Church, Va.	50	16.0
11	do	Charlottesville, Va.	54	55.5
11	do	Smithfield, Va.	50	6.0
17	Sweet corn	Burlington, N. J.	85	21.1
17	Field corn	Glasgow, Del.	97	19.5
18	Corn	Milford, Conn.	4	.0
21	Field corn	Hamburg, Pa.	68	41.1
20	do	Kingston, N. Y.	5	.0
21	do	Gettysburg, Pa.	100	15.0
21	do	Harrisburg, Pa.	100	2.0
Aug. 21	do	Franklin, Va.	100	40.0
21	do	Weldon, N. C.	27	18.6
30	do	Falls Church, Va.	100	29.0
Sept. 6	do	do	100	16.0

NUMBER OF INFESTED BEETLES LATE IN THE FALL

During the period of October 2 to November 22, several tests with beetles, involving 684 adults of *Chaetocnema pulicaria*, 39 of *C. denticulata*, and 48 of *Diabrotica duodecimpunctata*, were made in an effort to determine the percentage of these beetles that contained the bacterial wilt organism late in the fall of 1934, when they were about to hibernate. It was not always possible to obtain material for this work from areas immediately adjoining fields that had contained much of the disease during the previous summer. No *Aplanobacter stewarti* was isolated from *C. denticulata* and *D. duodecimpunctata*. Of the 684 adults of *C. pulicaria* tested, 90, or 13.1 percent, of the cultures yielded positive results. This probably was representative of the number of adult beetles of this species harboring

A. stewarti at this time. The collections of *C. pulicaria* used for these tests and the percentages known to harbor *A. stewarti* were as follows: Of 317 from Arlington Experiment Farm, 8.5 percent yielded *A. stewarti;* of 160 from Atchison County, Kans., 4.3 percent; of 110 from Platte County, Mo., 16.3 percent; and of 97 from Madison County, Ill., 39.1 percent.

HOST PLANTS OF APLANOBACTER STEWARTI

Until recently the only known host of the bacterial wilt organism, *Aplanobacter stewarti,* was the corn plant. This organism attacks

FIGURE 6.—Golden Bantam sweet corn leaves showing three stages in the development of bacterial wilt lesions developing from insect feeding injuries: *A*, Feeding injuries and beginning lesions; *B*, feeding injuries and young but well-developed lesions; *C*, old lesions.

several kinds of corn, but the most susceptible host is sweet corn (fig. 6). During September 1934 natural infection with *A. stewarti* was found on teosinte (*Euchlaena mexicana* Schrad.), under field conditions at Lanham, Md., and at Arlington Experiment Farm, Va. Infected plants were from seed from Florida and Guatemala. The

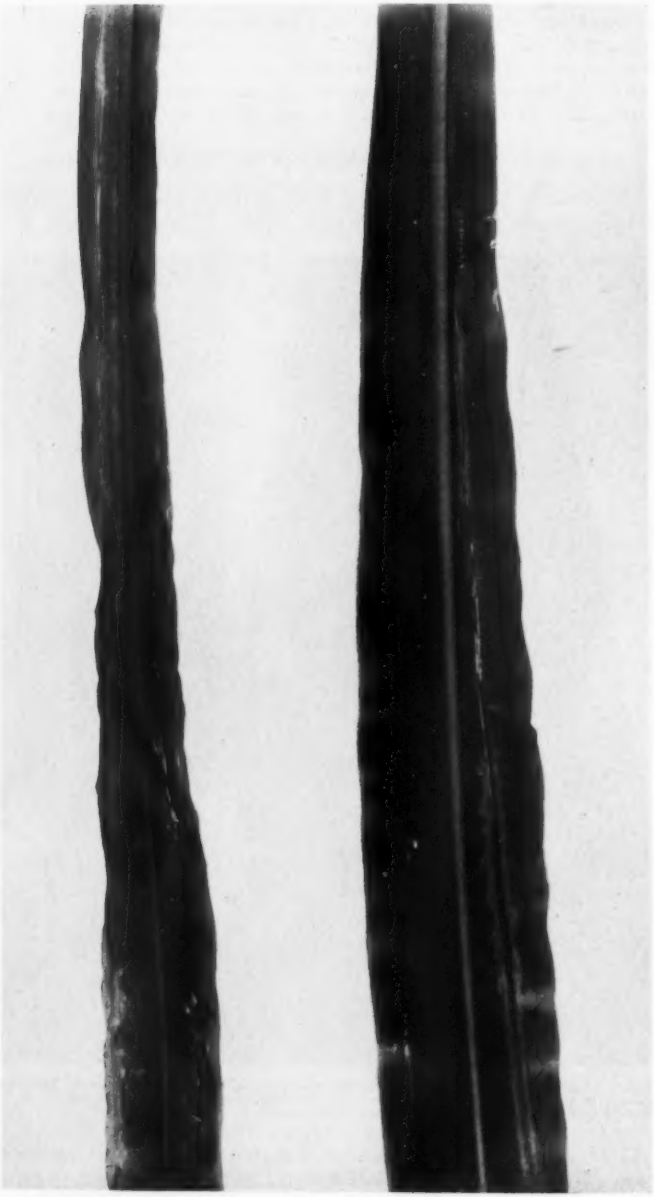

FIGURE 7.—Natural infection with *Aplanobacter stewarti* on leaves of teosinte (*Euchlaena mexican*) grown at Lanham, Md., from Florida seed. Photographed September 6, 1934.

lesions were similar to the leaf lesions on corn, but more limited than those occurring on susceptible varieties of corn. Isolations from these leaf lesions gave pure cultures of the typical yellow bacterium that produced wilt symptoms on Golden Bantam sweet corn in the greenhouse. A culture of *A. stewarti* isolated from the flea beetle *Chaetocnema pulicaria* produced long wilt lesions on teosinte leaves in the greenhouse and killed the plant. Adults of *C. pulicaria*, transferred from diseased corn to teosinte, produced wilt infection (fig. 7), and the wilt organism was reisolated in pure culture.

FIGURE 8.—Leaves of jobs-tears (*Coix* sp.) showing lesions of bacterial wilt inoculated by infective adults of *Chaetocnema pulicaria* in confinement.

Attempts to inoculate other plants, some of which are closely related to corn botanically, with pure cultures of *A. stewarti* by means of needle scratches and punctures, as well as by the feeding of adults of *C. pulicaria* which previously had been confined to diseased corn for a period of 5 days or longer, resulted in a failure to produce symptoms of the disease in any of these plants. The following species of plants were used in these studies: Goosegrass (*Eleusine indica* (L.) Gaertn.), smooth crabgrass (*Digitaria ischaemum* (Schreb.) Muhl.), eastern gamagrass (*Tripsacum dactyloides* (L.) L.), straw-colored cyperus (*Cyperus strigosus* L.), two varieties of sugarcane (*Saccharum officinarum* L.), Rimpan rye (*Secale cereale* L.), wheat (*Triticum aestivum*), Sudan grass (*Sorghum vulgare sudanense*), Johnson grass (*Sorghum halepense*), orchard grass (*Dactylis glomerata* L.), Kentucky bluegrass (*Poa pratensis* L.), and yellow bristlegrass (*Setaria lutescens*). More recent needle inoculations with cultures of *A. stewarti* have produced typical bacterial wilt lesions in

the leaves of jobs-tears (*Coix* sp.), and the wilt organism has been reisolated in pure culture from this plant. Jobs-tears has also been inoculated with *A. stewarti* (fig. 8) by means of adults of *C. pulicaria* which had been transferred from infected corn to this plant.

HABITS OF CHAETOCNEMA SPP.

CHAETOCNEMA PULICARIA MELSH.

Chaetocnema pulicaria was reported as early as 1891 by Forbes (*3, p. vii*) as severely injuring corn in Illinois, and the injury described on corn strongly suggests the presence of bacterial wilt, which was unknown at that time. This insect has attacked corn in injurious numbers frequently enough to be known commonly as the corn flea beetle. It hibernates in the adult stage and becomes active on warm days early in spring when the air temperature reaches approximately 70° F. at the surface of the soil. In 1933 adults of this species were first collected at Arlington Experiment Farm on April 2, and in 1934 on March 21. The adults attack the young corn as soon as it appears above the surface of the soil. The immature forms of this species probably develop in the soil, although they have not been definitely

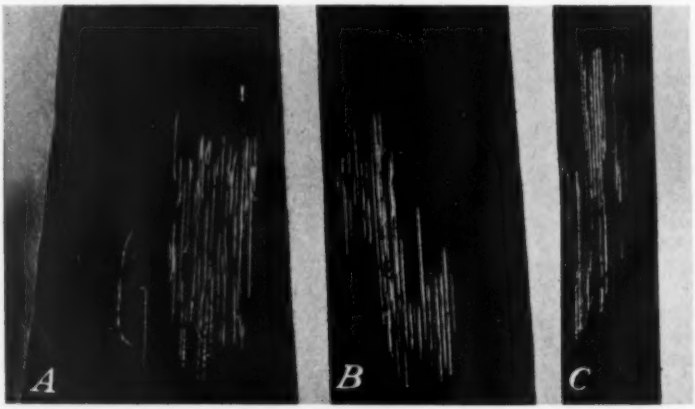

FIGURE 9.—Feeding by one adult of *Chaetocnema pulicaria* for 24 hours on sweet corn (*A*), Johnson grass (*B*), and *Cyperus* sp. (*C*).

identified in nature. The species is very small, the adults being 1.5 to 1.8 mm in length. They live for many months, are very active, and feed upon a wide variety of plants. Some adults have lived in the greenhouse at 65° F. from October to April. A few specimens passed through their life cycle on bluegrass in the greenhouse during the winter of 1934–35.

This species has been reported from most of the United States except the Northwest. Attempts to verify records of the reported occurrence in California have thus far been unsuccessful. Reports of the disease in California, therefore, suggest an insect vector other than those named in this paper, possibly the desert corn flea beetle, *Chaetocnema ectypa* Horn. Unpublished records on collections of insects made by airplane at Tallulah, La., by P. A. Glick during 1928–31 and made

available by him through the Division of Cotton Insect Investigations, Bureau of Entomology and Plant Quarantine, revealed the presence of *C. pulicaria* at altitudes ranging from 20 to 5,000 feet. This species was collected by airplane during nearly every month throughout the year at Tallulah. These data indicate that the species probably migrates or is carried in air currents for considerable distances. In two trap lights operated at Arlington Experiment Farm from May 6 to June 22 and from September 12 to December 5, 1934, one adult of *C. pulicaria* was collected November 3. The extreme activity of this insect was evidenced by the difficulty experienced in confining adults within cheesecloth cages even when a very fine mesh cloth was used. Field cages covered with similar cloth and used to exclude insects

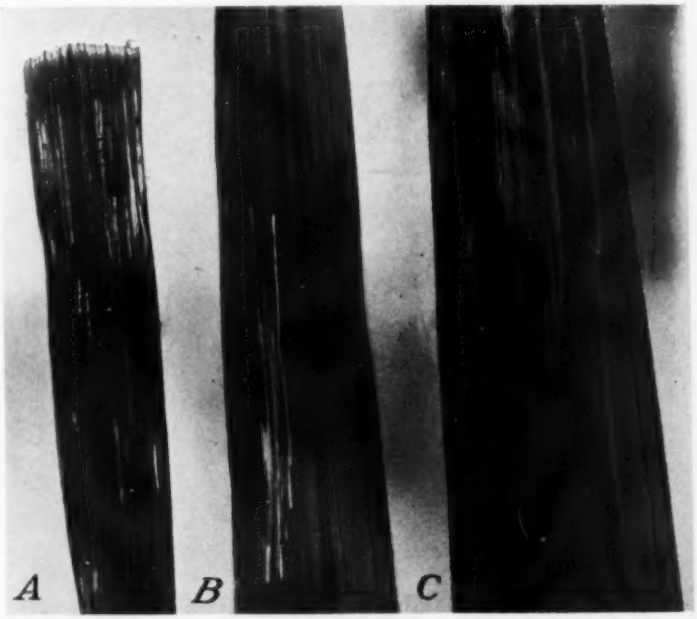

FIGURE 10.—Feeding by adults of *Chaetocnema pulicaria* on rye (*A*), teosinte (*Euchlaena mexicana*) (*B*), and eastern gamagrass (*Tripsacum dactyloides*) (*C*).

from hills of corn did not exclude this species unless the tacks holding the cloth were supplemented by strips of molding. From a collection of 50 adults of *C. pulicaria* taken from such cages on August 7, 34 (or 68 percent) yielded *Aplanobacter stewarti*, while a collection of 66 adults taken from Sudan grass at some distance away in the same field on the same afternoon yielded *A. stewarti* in only 8 instances.

The feeding habits of this species were studied on several host plants, and the amount and type of this feeding on the leaves of six species of plants are illustrated in figures 9 and 10. When the beetles were confined to corn in small cages, there was no apparent choice between feeding on the upper or on the lower surface of the leaves. In the field most of the feeding appeared to be on the upper surface. In

feeding, the adult always assumed a transverse position on the leaf and moved sidewise as it fed. It eats through to the epidermis on the opposite side and leaves a more or less transparent injury running parallel to the veins and appearing as a short, narrow, comparatively straight, white line.

Forty preliminary tests were made to determine the number of hours of feeding required for an adult of *Chaetocnema pulicaria* to inoculate healthy corn with the bacterial wilt organism, after it had fed at least 5 days on infected corn. Only negative results were obtained. These tests were carried on in the greenhouse in October 1934. Individual adults of *C. pulicaria* were confined to the leaves of corn plants in spring-clip cages (fig. 1) as follows: 10 for 2 hours, 10 for 6 hours, 10 for 8 hours, and 10 for 24 hours. Feeding apparently occurred in a normal manner and the plants grew slowly but normally; however, no lesions of the disease appeared on these plants.

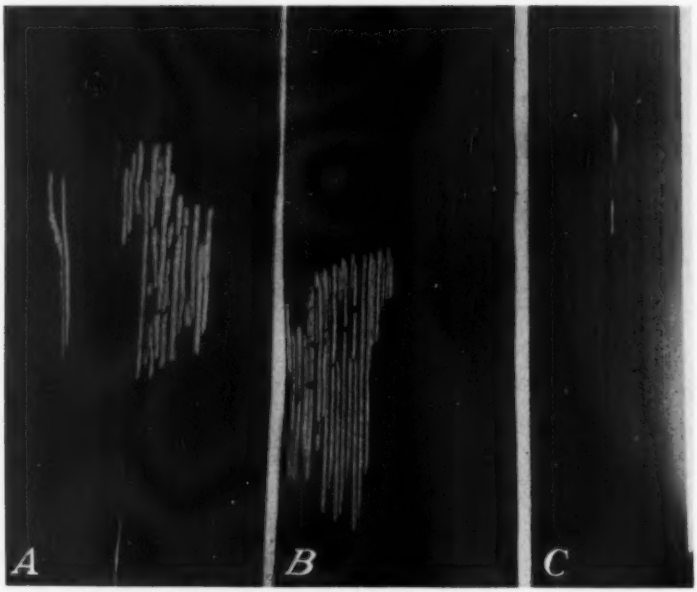

FIGURE 11.—Feeding by one adult of *Chaetocnema denticulata* for 24 hours on sweet corn (*A*), Johnson grass (*B*), and *Cyperus* sp. (*C*).

CHAETOCNEMA DENTICULATA (ILL.)

The adults of *Chaetocnema denticulata* are larger than those of *C. pulicaria*, ranging from 2.3 to 2.8 mm in length. Under field conditions at Arlington Experiment Farm during 1934 the species passed through at least two complete generations on Johnson grass. For the most part it was not so abundant as was *C. pulicaria* and fed much less on corn. The life cycle of a few individuals was completed under controlled conditions and extended over about 1 month. Under field conditions the eggs were found at the base of its host plant near the surface of the soil. The half-grown to full-grown

larvae are commonly found feeding on the tender unfurled leaves or on the developing seed head of Johnson grass. Pupation takes place in the soil, and the species overwinters in the adult stage. Feeding injuries on corn and Johnson grass are similar to those described for *C. pulicaria*, except that the green areas removed from the leaves are wider (fig. 11). The method of feeding appears to be identical with that of *C. pulicaria*.

CHAETOCNEMA CONFINIS CROTCH

Chaetocnema confinis has been reported as causing injury to corn similar to that caused by *C. pulicaria* (*4, pp. 110–111*). The present

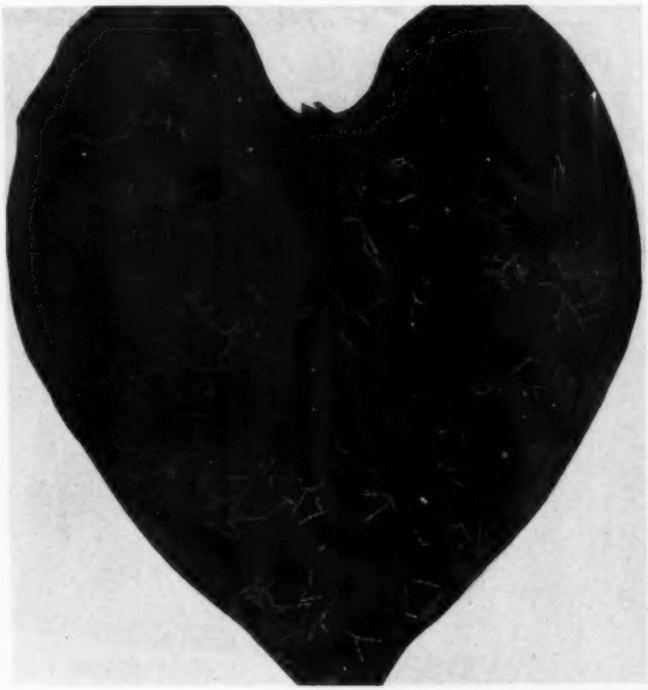

FIGURE 12.—Feeding injuries of *Chaetocnema confinis* to sweetpotato leaf. Note irregular paths of feeding.

writers' observations do not confirm this, although *C. confinis*, commonly known as the sweetpotato flea beetle, has often been collected on corn and Sudan grass when growing in proximity to convolvulaceous plants. A small number of feeding tests on corn under controlled conditions revealed no feeding injury in any way similar to that of *C. pulicaria* or *C. denticulata*. The adults of *C. confinis* appeared to die prematurely when confined to corn. On the other hand, the adults lived for long periods and fed considerably when confined to sweetpotato plants (fig. 12). This is an additional

reason for believing that the isolations of *Aplanobacter stewarti* obtained from *C. confinis*, as reported in table 1, may be due to misidentifications of the closely related species used.

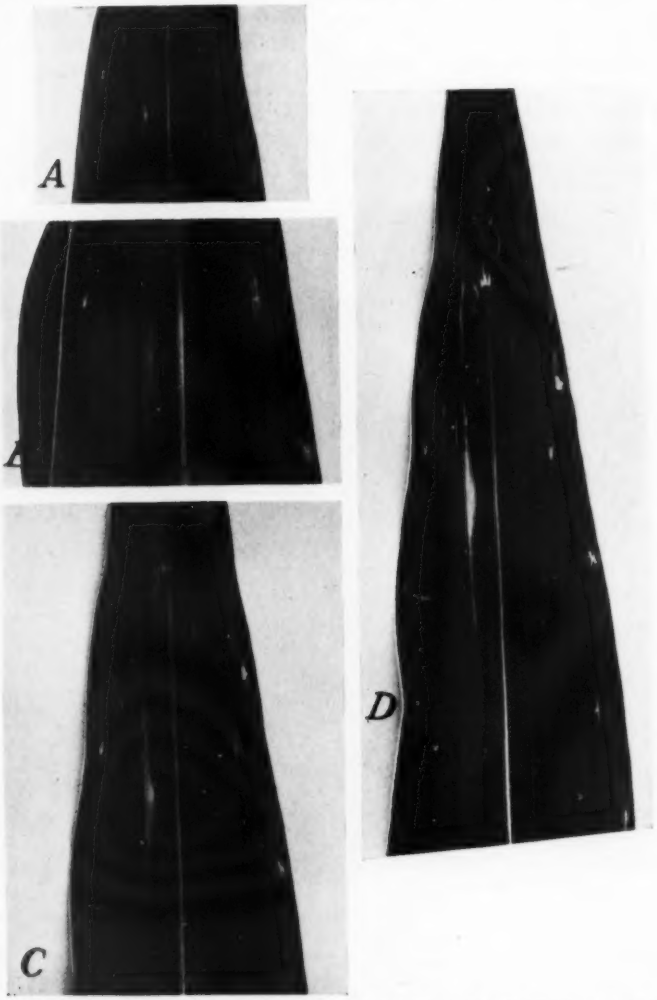

FIGURE 13.—Four stages in the development of a typical lesion of bacterial wilt following a small feeding injury to a sweet-corn leaf by *Chaetocnema pulicaria*, under field conditions. Photographed: *A*, August 1; *B*, August 6; *C*, August 10; *D*, August 13.

DEVELOPMENT OF LESIONS ON CORN

On August 17, 1933, a number of lesions of bacterial wilt, which started on the leaves of the corn at feeding places of flea beetles,

were measured to obtain definite data on the development of these secondary infections under field conditions on Golden Bantam corn grown from seed obtained from several localities. The lesions were marked with india ink, and each leaf was identified by means of a linen tag. Unusual, almost continuous, rainfall on August 21, 22, and 23 (5.88 inches on Aug. 23) interfered with making the measurements as planned. The final measurements were made August 26, and the results, presented in table 4, indicate the rapid development of the lesions.

TABLE 4.—*Development of lesions of Aplanobacter stewarti following feeding by Chaetocnema pulicaria on leaves of Golden Bantam corn at Arlington Experiment Farm, 1933*

Lot no.	Source of seed	Lesions measured	Aug. 17		Aug. 26		Average increase in length
			Average length	Average width	Average length	Average width	
		Number	*Mm*	*Mm*	*Mm*	*Mm*	*Percent*
1	Oregon	17	16.8	2.7	248.2	10.7	1,377.4
2	Idaho	9	21.1	2.7	313.3	11.0	1,384.8
3	Seed company in District of Columbia	7	12.4	2.8	138.5	6.2	1,016.9
4	Do	18	11.9	2.0	160.5	6.4	1,248.7

It will be noted from table 4 that the average increase in length of lesions in four lots during a 9-day period varied from approximately 10 to 14 times the original size.

During 1934 a few similar measurements were made of lesions on leaves of a susceptible variety of corn, and four photographs were made of what appeared to be a typical lesion as it developed in the field (fig. 13). The measurements of this lesion are given in table 5.

TABLE 5.—*Measurements of a lesion of Aplanobacter stewarti on a leaf of sweet corn, 1934*

Date [1]	Length	Width	Date	Length	Width
	Mm	*Mm*		*Mm*	*Mm*
Aug. 1	7	1	Aug. 8	44	4
Aug. 3	10	2	Aug. 10	115	4
Aug. 6	38	4	Aug. 13	160	5

[1] The lesion was photographed on Aug. 1, 6, 10, and 13. On Aug. 17 the lesion coalesced with others.

MAXIMUM INFESTATION OF CHAETOCNEMA PULICARIA AND C. DENTICULATA BY APLANOBACTER STEWARTI

Tests were made to determine the percentage of adults of *Chaetocnema pulicaria* from which *Aplanobacter stewarti* could be isolated after being confined to infected corn in cages in the outdoor insectary for a minimum of 5 days. These insects were sterilized externally. The results are shown in table 6.

TABLE 6.—*Percentage of Chaetocnema pulicaria containing Aplanobacter stewarti after confinement on infected corn for 5 days, 1934*

Date cultured	Adults tested individually	Isolations of *A. stewarti*	Proportion containing *A. stewarti*
	Number	*Number*	*Percent*
Aug. 8	50	34	68.0
Aug. 23	75	23	30.7
Sept. 18	102	69	67.6
Sept. 28	115	64	55.7
Total	342	190	
Average (weighted)			55.6

It will be noted from the table that only from 30.7 to 68 percent, or an average of 55.6 percent, of the adults of *Chaetocnema pulicaria* yielded *Aplanobacter stewarti* in these tests, even after these beetles had been confined to infected corn in cages for a minimum of 5 days. Another lot of 78 adults of *C. pulicaria*, tested at the same time by placing the surface-treated uncrushed insects on agar plates, yielded no colonies of *A. stewarti*. Of most interest in these latter tests was the fact that some of these beetles survived the entire process. Of 23 adults of *C. denticulata* confined to infected corn in cages for a minimum of 5 days, 11, or 47.8 percent, yielded colonies of *A. stewarti*. These percentages of beetles containing the wilt organism are unexpectedly low when compared with a maximum of 75 out of 100 adults of *C. pulicaria* that were found to contain *A. stewarti* when collected from plots of infected sweet corn under field conditions July 9, 1934, at Arlington Experiment Farm.

RETENTION OF APLANOBACTER STEWARTI BY THE BEETLES

Two series of experiments were conducted with *Chaetocnema pulicaria* and *C. denticulata* in order to determine how long adults of these beetles remained infective after being confined in cages for 5 days or more with diseased plants. The plants placed in the test cages were replaced by other healthy plants at intervals of not more than 5 days in order to prevent the possibility of the beetles becoming reinfected from the plants which they had inoculated. This possibility was considered very remote because of the slow development of the wilt symptoms under the conditions of these experiments. In both series of experiments the beetles of each lot apparently remained infective for approximately the entire period of their life.

In tests conducted in 1933 it was noted that some specimens of *Chaetocnema pulicaria* remained infective for a period of 22 days, i. e., they were able to inoculate susceptible sweet corn with the organism 22 days after having fed on infected corn. This observation suggested the tests made with overwintered adults in April 1934 which led to the discovery that *Aplanobacter stewarti* overwinters in the bodies of these flea beetles.

Tests made during 1934 indicated that one lot of 25 adults of *Chaetocnema denticulata* inoculated healthy corn plants with *Aplanobacter stewarti* from September 14 to October 20 inclusive (all of these adults died by Nov. 14), or for a period of at least 37 days.

In similar tests with a lot of 25 adults of *C. pulicaria*, healthy corn plants were inoculated from September 26 to November 13 inclusive (all of these adults died by Nov. 26), or for a period of at least 49 days. Similar tests made with adults of *Diabrotica duodecimpunctata* during February 1934 indicated that the adults of this species were able to transmit the bacterial wilt organism from diseased to healthy corn plants after being removed from the diseased plants from 5 to 9 days. Externally sterilized adults of this species which, after feeding several days on infected corn plants, had fed on healthy plants 6 and 15 days, respectively, did not yield the wilt organism when crushed in sterile beef peptone broth and plated in the usual manner.

BACTERIAL WILT AND ITS INSECT VECTORS IN RELATION TO WINTER TEMPERATURES

The association of warm winters with outbreaks of bacterial wilt has frequently been commented upon. Stevens [8] concluded that when the average winter temperatures are high, bacterial wilt has shown a tendency to increase, and vice versa. He reported that no obvious relation appears between the incidence of the disease and the total rainfall during any season, nor between the incidence of the disease and the temperature during spring, summer, or fall, although he stated that one may well exist. He established temperature indices for the winter months (sum of the mean temperatures of December, January, and February) and according to the data presented suggested that the disease will usually be absent in the Northeastern States following a winter with an index below 90° and present in destructive amounts following a winter with an index above 100°. The unusual abundance and distribution of this disease from 1929 to 1933 followed a series of mild winters. The amount of bacterial wilt in 1934 was much reduced following the low temperatures of the previous winter. This was particularly true in eastern New York and in New England, where the known insect vectors were almost entirely absent during 1934. The relationship of winter temperatures to the abundance and distribution of the insects which overwinter and transmit *Aplanobacter stewarti* may be very significant and has received some attention. *Chaetocnema pulicaria* was not found abundantly north of central Pennsylvania during 1934. *A. stewarti* was isolated from this insect from all localities where this beetle was found abundantly.

The effect of the extensive use of resistant varieties of sweet corn on the amount of bacterial wilt is a factor that should not be overlooked in predicting abundance of this disease. The problem is doubtless further complicated by lack of information on the abundance of the insect vectors in which the organism of the disease overwinters. The relative abundance and infectivity of the hibernating insect vectors may be of extreme importance in predicting the appearance of bacterial wilt of corn in destructive abundance.

DISCUSSION

Since *Aplanobacter stewarti* has been known to overwinter in seed only to a limited extent (2 to 13 percent) and there is no direct

[8] STEVENS, N. E. STEWART'S DISEASE IN RELATION TO WINTER TEMPERATURES. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repts. 18: 141-149, illus. 1934. [Mimeographed.]

evidence of its overwintering in naturally infested soil in the field, the finding of this organism overwintering in a virulent condition in the corn flea beetle, under natural conditions, offers a satisfactory explanation as to the source of early spring infections. Furthermore, the high percentages of beetles infested with the organism throughout the corn-growing season readily account for the midseason spread of the disease.

The problem of determining the part played by insects in the overwintering and dissemination of bacterial wilt of corn is a very large one. Neiswander (*6*), in his paper on the sources of American corn insects, reports that 352 species of insects have been recorded in the literature as attacking the corn plant. He suggests that some of these records are probably obsolete and have never been verified by later observations. However, he lists 166 species as significant corn insects. How many of these may be carriers of the wilt organism remains to be determined. Isolations for *Aplanobacter stewarti* were made during 1934 from 40 species of insects belonging to 33 genera. Eight species representing six genera yielded *A. stewarti*. Only the corn flea beetle (*Chaetocnema pulicaria*) and the toothed flea beetle (*C. denticulata*) appeared to be of importance in the dissemination of the disease. Much further work is necessary in order to determine whether the other six species involved are normally vectors of *A. stewarti*.

Transmission of bacterial wilt from infected to healthy corn was effected by means of adults of the spotted cucumber beetle (*Diabrotica duodecimpunctata*), but none of the 178 adults of this species collected throughout the season under field conditions yielded *Aplanobacter stewarti*.

Collections of *Chaetocnema pulicaria* from or near plantings of sweet corn at Arlington Experiment Farm at intervals of 7 to 14 days yielded an increasing number of isolations of *Aplanobacter stewarti* up to July 9, on which date a maximum of 75 out of 100 individuals yielded this organism. After this date the number again decreased. The maximum proportion of *C. pulicaria* that yielded *A. stewarti* after being confined to infected corn in cages for at least 5 days was 68 percent, and the average of the 342 such beetles tested was 55.5 percent. It seemed that this percentage should have been higher under these circumstances. The isolations of *A. stewarti* obtained from *C. pulicaria* collected from sweet corn at Arlington Experiment Farm during the period May 14 to September 19 averaged 40.3 percent, whereas similar collections from other host plants (including field corn) during the same period yielded an average of 12.1 percent. In collections of *C. pulicaria* from various localities throughout the season of 1934, from 0 to 75 percent of the individuals in each collection yielded *A. stewarti*.

Very rapid development of lesions of bacterial wilt following feeding on the corn leaves by the corn flea beetle was observed under field conditions. The average increase in length of lesions in four lots during a 9-day period varied from approximately 10 to 14 times the original size. The increase in size of the wilt lesions was greater on the Golden Bantam corn grown from seed produced in Oregon and Idaho than on corn grown from seed produced in wilt-infected areas. Plants produced from seed grown in a section where the disease does not occur are often more susceptible to bacterial wilt than are plants from seed grown in wilt-infected areas.

SUMMARY

Bacterial wilt of corn is not transmitted through the soil; this finding confirms the results obtained by earlier workers.

Direct transmission of bacterial wilt from infected to healthy corn was effected by means of three species of beetles, *Chaetocnema pulicaria*, *C. denticulata*, and *Diabrotica duodecimpunctata*, thus verifying earlier work of this type. Similar tests with 13 other species of insects yielded only negative results.

Aplanobacter stewarti was found to pass the winter in a virulent condition in the bodies of the hibernating adults of the corn flea beetle, *Chaetocnema pulicaria*. Approximately 19 percent of 175 of these overwintering beetles which were tested during April 1934 yielded *A. stewarti*. Young corn plants are inoculated with bacterial wilt in the spring by the feeding of these beetles, and this appears to be the chief source of early infection each year.

A total of 7,338 insects, representing 40 species in 33 genera, collected on or near corn infected with bacterial wilt, during the corn-growing season in 1934, were tested for the presence of *Aplanobacter stewarti*, and eight species yielded this organism. A total of 3,977 isolations were made, 1,464 of which were suspected of containing *A. stewarti* and were tested by inoculation on corn, 953 yielding positive results.

Of 908 adults of *Chaetocnema pulicaria* collected from sweet corn at Arlington Experiment Farm, during the period May 14 to September 19, an average of 40.3 percent yielded *Aplanobacter stewarti*, whereas of 669 adults of the same species collected during the same period from various other host plants (including field corn) at the same place an average of 12.1 percent gave *A. stewarti*. The maximum proportion of isolations of *A. stewarti* obtained from adults of *C. pulicaria* was 75 percent.

Collections of adults of *Chaetocnema pulicaria* from 12 different host plants and from 29 different localities yielded *Aplanobacter stewarti* in from 0 to 75 percent of the individuals in each collection.

Of the 684 adults of *Chaetocnema pulicaria* collected and tested late in the fall when they were about to hibernate, 13.1 percent yielded *Aplanobacter stewarti*.

In addition to corn, teosinte (*Euchlaena mexicana*) and jobs-tears (*Coix* sp.) were found to be hosts of *Aplanobacter stewarti*. Infection was obtained both by needle inoculations and through the feeding of infective flea beetles, *Chaetocnema pulicaria*. Tests with several other grasses yielded only negative results.

The length of bacterial wilt lesions, following feeding injury by adults of *Chaetocnema pulicaria* on corn in the field, was found to increase approximately 10 to 14 times the original size during a 9-day period in 1933.

Compared with 1932 and 1933, the amount of bacterial wilt in eastern New York and in New England was much reduced in 1934 following the low temperatures of the previous winter. *Chaetocnema pulicaria* was not found abundantly north of central Pennsylvania during 1934. The relationship of winter temperatures to the abundance and distribution of the insects which overwinter and disseminate *Aplanobacter stewarti* may be very significant in predicting the appearance of bacterial wilt of corn in destructive abundance.

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GROWTH INHIBITION IN THE POTATO CAUSED BY A GAS EMANATING FROM APPLES[1]

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INTRODUCTION

When the potato, *Solanum tuberosum* L., is confined with gases emanating from apple fruits its growth is arrested. This reaction was first noted in investigations of temperature effects on the pathogenicity of *Rhizoctonia* to the potato. All the potato seed pieces kept in incubators in a cold-storage room containing apples produced small, abnormal sprouts (*7*).[3] Subsequent investigations have proved that a volatile substance normally produced by ripe apple fruits was the cause of this growth inhibition.

The object of this paper is to describe the morphological and physiological effects on the potato of the growth-inhibiting gas from apples, and to present information concerning the characteristics and chemical identity of this substance.

Sound, nondormant potato tubers, growing potato plants, and ripe apples (fruits of *Malus malus*) were used in these studies. The potatoes, usually cut into seed pieces, were planted in moist soil, sawdust, or sphagnum, under conditions favorable for their growth. The planted seed pieces or the growing plants, together with a few apples, were placed in a closed container in order to provide a sufficient concentration of the growth-inhibitory gas from the apples, but the fruits were never in actual contact with the potatoes. As a rule, containers with a capacity of approximately 2 cubic feet or less were used, and generally the tests were made in the greenhouse. The controls consisted of potatoes growing under similar environmental conditions except that apples were omitted from the container.

MORPHOLOGICAL CHANGES IN THE POTATO PLANT DUE TO THE PRESENCE OF APPLES

EFFECT ON THE SPROUTS

The volatile substance from apple fruits affects the potato plant by markedly limiting the growth of the stem and roots. The limited sprout growth develops radially approximately as fast as longitudinally, and there results a tuber like structure closely appressed to the potato eye (fig. 1, *A*, and *B*). Radial enlargement is most rapid near the apex, where the tissues are still meristematic. Rupturing of the outer layers near the apical end frequently occurs (fig. 2, *B*).

Abnormally thickened and fleshy rootlets sometimes develop from the sprouting eyes (fig. 3, *C*). When normal potato roots are exposed to the gas from apples, apical growth ceases, and abnormal enlargement in diameter occurs near the tip (fig. 2, *B*).

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[2] The studies of the chemical nature and characteristics of the growth-inhibitory gas were performed with the advice of Dr. H. N. Barham, of the Kansas State College of Agriculture and Applied Science, Department of Chemistry.

[3] Reference is made by number (italic) to Literature Cited, p. 626.

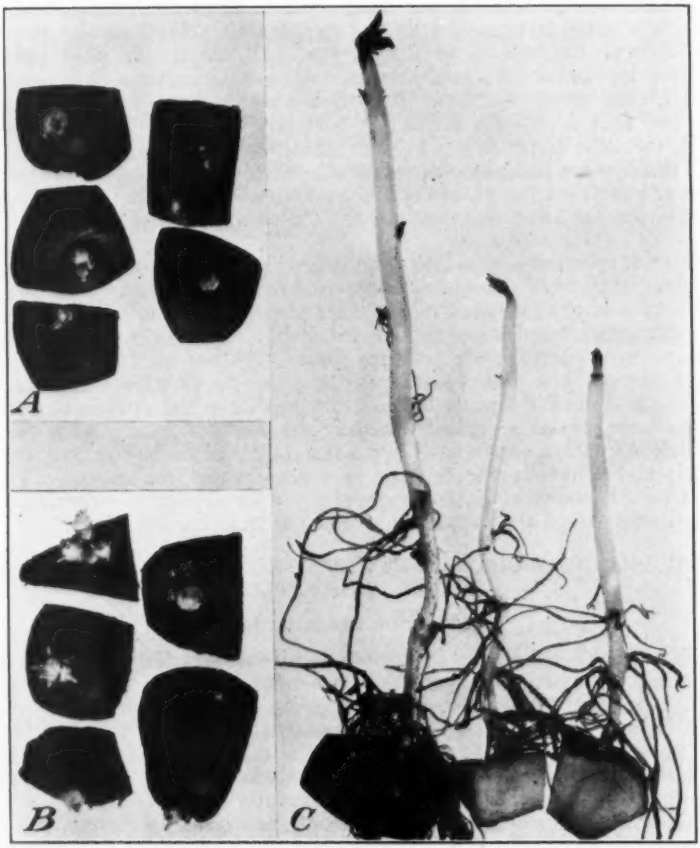

FIGURE 1.—Sprout development in potatoes exposed to growth-inhibiting gas: *A*, Stunted, abnormal sprouts from seed pieces confined 10 days with apples; *B*, stunted, abnormal sprouts from seed pieces confined 10 days with hawthorn fruits; *C*, normal sprouts from control seed pieces.

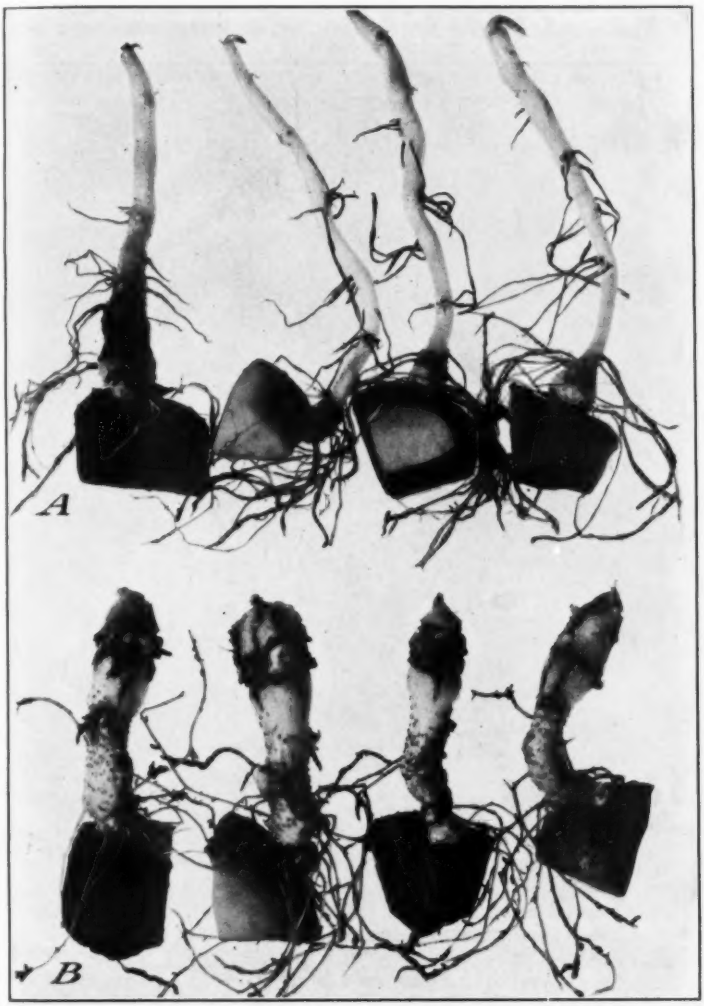

FIGURE 2.—Duration of the effect of growth-inhibiting gas on sprout development: *A*, Sprouts grown 7 days with apples and then 7 days without apples; *B*, sprouts grown 7 days without apples and then 7 days with apples.

A test was made early in these investigations to determine whether the conditions under which potatoes had previously been kept had any effect upon their response to the growth-inhibiting gas from apples and whether different varieties of potatoes reacted similarly when confined with apples. Tubers of Early Ohio and Irish Cobbler from Minnesota, Netted Gem from Idaho, Spaulding Rose No. 4

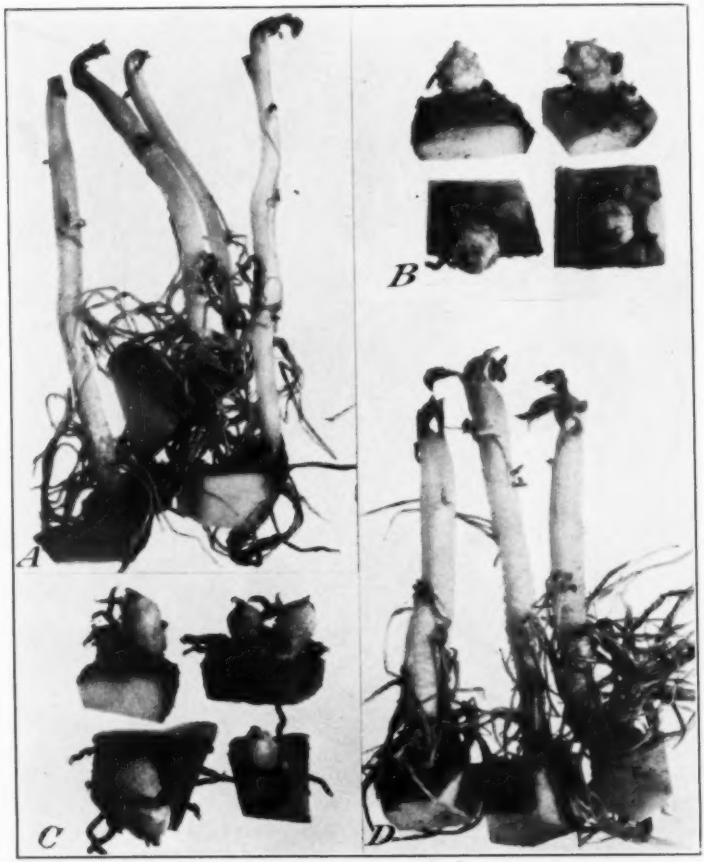

FIGURE 3.—*A*, Normal sprouts developed from tubers confined 14 days with cider; *B*, abnormal sprouts developed from tubers confined 14 days with pressed pulp of apple fruits; *C*, abnormal sprouts developed from tubers confined 14 days with sound apples; *D*, normal sprouts from the controls.

from Maine, Bliss Triumph from Montana, White Triumph from western Kansas, and an undetermined variety from Oregon were tested. All of these lots of potatoes produced the characteristic abnormal growth, and the responses of the different varieties were similar.

The gas from apples may suppress the bud dominance in the tuber eye and thus make it possible for buds to develop that would normally

remain dormant and for multiple sprouting to result. This was particularly noticeable in the Triumph and Early Ohio varieties, in which from 7 to 12 buds started growing from numerous single eyes. Single buds, however, also developed from some of the eyes of these same tubers, but the potatoes that were confined with apples produced a greater number of sprouts on an average than did the control tubers.

A test was made to determine whether the effect of apples on bud dominance persists after the tubers are planted in the field. Forty Irish Cobbler and 40 Bliss Triumph tubers that had been held in storage with apples were cut into seed pieces and then planted. They

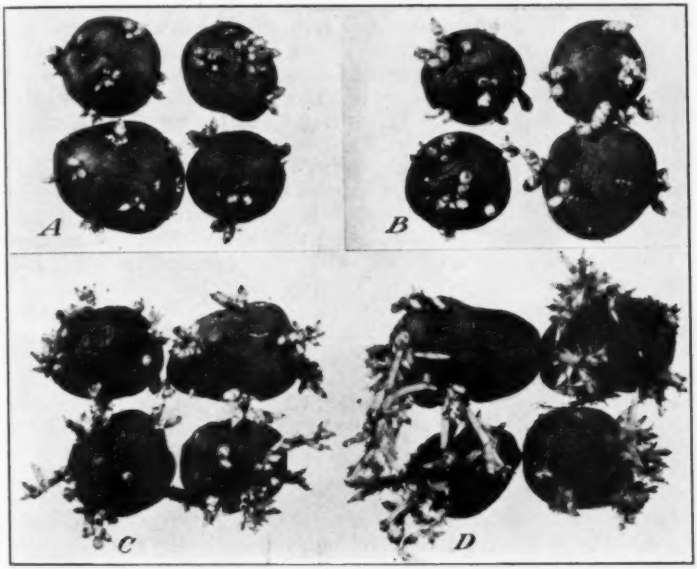

FIGURE 4.—Preservative effect of apples on 60-pound lots of potatoes stored with apples from January until June in 5-cubic-foot containers: *A*, Tubers stored with 10 pounds of apples; *B*, tubers stored with 5 pounds of apples; *C*, tubers stored with 1 pound of apples; *D*, control tubers.

produced 198 and 171 sprouts, respectively, while the 40 control tubers of each of these varieties produced 159 and 135 sprouts, respectively. In this test the Irish Cobbler and Bliss Triumph tubers that had been held with apples both produced approximately 25 percent more sprouts than did the control tubers.

When tubers were planted in moist soil under favorable growing conditions and confined with apples, abnormal sprout development was more rapid than was the case when the tubers were kept dry. Figure 3, *C*, illustrates the sprouts that developed in 2 weeks from treated tubers planted in moist soil, and figure 4, *A*, illustrates the smaller sprouts produced by tubers held with apples from January until June in a commercial potato-storage house.

Duration of the Effect

The inhibition of normal growth apparently becomes operative as soon as potato plants are placed under the influence of a sufficient concentration of the growth-inhibiting gas. When affected potatoes are removed from its presence, normal growth is apparently resumed immediately.

Pots containing sprouting seed pieces planted in soil were placed in two closed cans, one of which contained apples. At the end of 7 days the seed pieces in the can containing apples had produced stunted sprouts, whereas those not exposed to apple gas had produced normal sprouts averaging more than 5 cm in length.

The two lots of seed pieces were interchanged; those that during the first 7 days of the test had produced normal sprouts were placed in the container with apples, and those grown in that container the first 7 days were transferred to the container in which there were no apples. During the next 7 days the seed pieces in the container with the apples made no appreciable growth in length, but their stems enlarged radially 300 percent (fig. 2, *B*). Radial enlargement was most rapid near the apex, where the tissues were still meristematic. Frequently the outer layers ruptured near the apical end.

Linear growth started immediately in the sprouts stunted the first 7 days of the test (fig. 2, *A*) and averaged more than 8 cm during the 7 days they were in the container without apples. The basal radial enlargements were still evident at the end of the second week.

Seed pieces which had been planted in soil and confined with apples for 2 weeks were planted at a depth of 3 inches in a soil bed, and a like number of seed pieces which for the preceding 2 weeks had been planted in soil but not confined with apples were planted alongside at the same depth. The short, thickened sprouts of the seed pieces confined with apples were not removed. Only sprouts that were 5 mm or less in length were allowed to remain on the seed pieces that had not been confined with apples. No significant difference was noted in the time required for sprout emergence from the two lots. This indicates that the effect of the growth-inhibiting substance did not persist after the potatoes were removed from this gas.

Effect on the Green Plant

Above-ground portions of the potato plant are definitely affected by the growth-inhibiting gas from apples. Normal growth ceases, the leaf blades, leaf petioles, and stems become abnormally rigid, and the older leaves bend downward as though wilted. Crocker (*4*) noted this effect, known as epinasty, on certain other plants when they were subjected to the gases ethylene, acetylene, propylene, butylene, and carbon monoxide.

The drooping of the potato petioles began within an hour after the plants were placed under a bell jar with apples, and the response was very noticeable after 2 or more hours. The time lag of an hour before visible symptoms appeared was probably due to the fact that the necessary dosage of the inhibitory gas had to accumulate and that it took the plant some time to react.

The potato plants were allowed to remain under bell jars for several days (fig. 5) in this experiment. After 3 or 4 days the older leaves of plants exposed to the apple gas became chlorotic. This was followed

by the loss of all green color, after which the petioles became detached by abscission. The very young leaves at the tip of the stem remained green and in an upright position but became rigid and did not grow perceptibly. While terminal growth of the plants held with apples was practically stopped, there occurred an abnormal radial growth or thickening of the stems and of the petioles of the partly developed leaves that remained attached. This thickening of the leaf petioles was greatest at the axils. Control plants held under bell jars without apples produced a normal growth.

EFFECT ON STORED POTATOES

Potatoes were stored with apples in boxes with a capacity of 5 cubic feet from January until early summer in order to obtain information on the effect of the gases from this fruit in preserving the tubers.

FIGURE 5.—Effect of the growth-inhibiting gas from apples on the potato plant: *A*, Control plant held 2 weeks under bell jar; *B*, potato plant confined 2 weeks with apples. The oldest leaves of *B* became chlorotic and died; the younger leaves curved downward, became rigid, and the petioles enlarged radially, especially at the axils. The stem also became thickened.

This experiment was first made in 1933 and was repeated in 1934. The storage boxes, which were not completely closed in order to allow ventilation, were held in a commercial potato-storage house at Topeka, Kans., which was kept at approximately 10° C. until March, after which the temperature rose because of the warmer weather, as artificial cooling facilities were not available.

Sixty pounds of sound Irish Cobbler potatoes were placed in each of four of the boxes on January 29, 1933, and to three of them were added respectively 1, 5, and 10 pounds of Winesap apples. The potatoes in a fourth box containing no apples served as a control. The test was completed June 24, at which time the four lots of potatoes were weighed, and final observations were made on their condition.

The tubers that were stored with the 5-pound and the 10-pound lots of apples developed the abnormal sprout growth that is induced by the gas from apples. They had remained firm, and none had decayed.

Inhibition of sprouting was most pronounced in the potatoes that were stored with 10 pounds of apples, and these tubers were in the best state of preservation, as is indicated in figure 4, *A*. The potatoes that were stored with 1 pound of apples produced sprouts that exhibited an unmistakable growth inhibition, although they resembled the controls closely. Only two potatoes had decayed, but the tubers were shriveled and in poor condition (fig. 4, *C*). The control tubers (fig. 4, *D*), on the other hand, had sprouted profusely, and were decidedly shriveled and in poor condition, and a few had decayed. The sprout growth from the control tubers was abnormally branched, and many of these sprouts, particularly in the lower layers of tubers, were dead. This condition was probably due to the lack of ventilation in the boxes. The loss in weight of the control tubers, after the sprouts were removed, was 8.75 pounds, and the loss in weight of the tubers that were held with 1, 5, and 10 pounds of apples was respectively 8.5, 7.25, and 4.75 pounds.

The potatoes that had been stored with 10 pounds of apples tasted sweeter than did the control tubers, indicating that more of the starch had been hydrolyzed to sugars. This effect is of considerable importance in regard to the practicability of using the growth-inhibiting gas from apples for preserving stored potatoes.

The storage test was repeated in 1934, but the quantities of apples in the three boxes were increased to 5, 10, and 20 pounds. The test was started in January and completed in June, and the results were similar to those obtained in 1933. The tubers that were confined with apples produced only a limited sprout growth, and they were better preserved than were the control tubers. A considerable number of tubers, however, were decayed, and comparative weight decreases which were due to respiration and evaporation could not be obtained. The state of preservation and the sprout development of the potatoes stored with the 10-pound and with the 20-pound lots of apples were similar. This suggests that under the conditions of these storage tests, 10 pounds of apples supplied to 60 pounds of potatoes a sufficient concentration of the growth-inhibiting gas for maximum prevention of sprouting.

In order to learn what effect the volatile gas from apples has on seed potatoes, tubers of the varieties Irish Cobbler and Bliss Triumph were planted in the field after they had been confined with apples for 60 days. Untreated tubers were planted as controls. The time required for the emergence of sprouts was similar for both the untreated and treated lots. The treated Cobbler and Triumph tubers produced respectively 25 and 27 percent more sprouts than did the control plants, but the yields were similar. The increased number of sprouts from the treated tubers is evidently an effect of the gas from apples, which causes bud dominance in the potato eye to be overcome. This test indicated that potatoes to be used for planting may be preserved with the growth-inhibiting gas from apples without harmful effects to their productivity.

SOURCE OF THE GROWTH-INHIBITING GAS

All the apple varieties that were tested caused growth inhibition of the potato, and this characteristic appears to be common to all ripe apples. Abnormal growth response of potato sprouts were obtained with Winesap, Stayman Winesap, Jonathan, Ben Davis, Gano, Delicious, Rome Beauty, and Yates apples.

Certain other fruits and vegetables were kept in closed containers with sprouting potatoes in order to ascertain whether, like the apple, they produce a growth-inhibiting gas. An inhibited sprout growth was produced by pears, *Pyrus communis* (varieties Bosc and Kieffer), and by the fruits of the hawthorn, *Crataegus crus-galli* (fig. 1, *B*). Normal growth resulted when the planted tubers were subjected to the gases produced by bananas, onions, oranges, or sweetpotatoes.

Immature apples did not cause growth inhibition in the potato plant, and it appears that the growth-inhibiting substance is not produced by such fruits. When the immature apples were held, however, until they had ripened and were again placed in an enclosed chamber with potatoes, they caused the characteristic growth inhibition.

It was noted that the growth-inhibiting substance is produced in smaller quantities by overripe fruits and not at all by decayed fruit tissue. When sound but overripe pear and hawthorn fruits were confined with potatoes, the resultant sprout growth was thickened, elongated slowly, and was more or less intermediate between the normal and the abnormal. A similar but less definite response was noted in overripe apples. When potatoes planted in soil were placed in a chamber with decaying apples, abnormal sprouting developed as long as healthy apple tissue was present, but normal growth began when the apples were entirely decayed.

Studies were made to determine what tissues in the apple produce the growth-inhibiting gas. Freshly isolated mesocarp, endocarp, skin, and seeds were renewed daily in closed chambers containing sprouting potatoes. Inhibited growth similar to that occurring when the whole fruit was used resulted in the potatoes that were confined with the mesocarp and with the endocarp tissues. Abnormal growth of sprouts did not occur on the potatoes held in containers with the skin or the seeds of apples; and it appears, therefore, that these portions of the apple do not produce the growth-inhibiting gas.

Growth inhibition was not obtained from potatoes that were confined with cider but did result when the potatoes were confined with pressed apple pulp. Ground apples were pressed in a hydraulic press under 19,000 pounds pressure, and the juice and pulp fractions were placed in separate closed chambers containing planted potatoes. Each portion of juice and pulp was allowed to remain in the container for a day and was then replaced by a newly prepared portion. The normal sprout growth from the potatoes confined with the cider and the abnormal growth from those that were held with the pulp is shown in figure 3.

Steam distillate from crushed apples was confined with sprouting potatoes but did not prevent normal sprout production. Newly prepared portions of the distillate were supplied daily, and each portion was allowed to remain but 1 day with the potatoes. The growth-inhibiting substance was evidently not concentrated in the distillate from the fruits.

CHANGES IN NORMAL PHYSIOLOGICAL PROCESSES

RESPIRATION

Measurements were made on the intensity of respiration from sprouting potato tubers that were growing in normal atmosphere and from tubers that were held in a similar environment with the excep-

tion that the atmosphere contained the volatile emanations from apples.

The two lots of tubers were each confined in an enclosed chamber through which a stream of air was constantly drawn. The air supplied to the control-tuber lot was obtained directly from the laboratory and permitted normal sprout growth. The air supplied to the other lot of potatoes was charged with the volatile gases from apples by drawing air from the laboratory through a cylinder 25 cm in diameter and 75 cm long, filled with ripe apples. These potatoes produced the characteristic abnormal sprouting that results when growing potatoes are confined with the growth-inhibiting gas from apples.

A measure of the intensity of respiration of the experimental potatoes was obtained from the increase in weight of soda lime after the salt had absorbed the carbon dioxide respired from weighed lots of the potatoes for measured periods of time. Atmospheric carbon dioxide was removed from the stream of air supplied to the growing chambers by first passing the air through soda lime. Calcium chloride was employed to dry the air before it entered the soda-lime towers.

Sound, uncut tubers were used, and their weight was recorded at the beginning of the test when they were placed in the enclosed growing chambers. Not less than 1,000-g samples of tubers were used. The different tests, which were made at laboratory temperatures of approximately 20° to 25° C., varied in length from 10.5 to 21 days. A moist atmosphere was provided for certain lots by packing the tubers in moistened sphagnum, while other lots were kept in a dry atmosphere.

Four series of tests on respiration intensity were made, and the results are recorded in table 1. This table records the weight increases of the soda lime from carbon dioxide respired by the experimental tubers. In order that comparisons may be readily seen, there is given in the table the computed weight of carbon dioxide respired from 1,000 g of tubers during a period of 21 days. The percentage increase of carbon dioxide respiration from the affected tubers in excess of that from the nonaffected tubers is also given.

TABLE 1.—*Effect of the growth-inhibiting gas from apples upon respiration from sprouting potato tubers*

Date	Potatoes held moist or dry	Growth-inhibiting gas absent				Growth-inhibiting gas present			Increase of carbon dioxide in abnormal potatoes
		Length of test	Weight of tubers	Carbon dioxide respired	Carbon dioxide respired per 1,000 g potatoes per 21 days	Weight of tubers	Carbon dioxide respired	Carbon dioxide respired per 1,000 g potatoes per 21 days	
		Hours	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
October 1932	Dry	504	1,191	12.79	10.74	1,247	29.64	23.77	
						1,190	20.75	17.44	92
	Moist	504	1,290	10.48	8.12	1,180	20.52	17.40	
						1,205	14.07	11.68	79
January 1933	Dry	504	1,045	5.47	5.23	1,050	17.15	16.33	
						1,050	10.32	9.83	150
	Moist	504	1,045	6.29	6.02	1,045	13.41	12.83	
						1,050	9.88	9.41	85
January 1934	do	253	1,000	2.46	4.91	1,000	5.29	10.54	115
February 1934	Dry	310	1,030	3.23	5.10	1,040	9.20	14.38	182
		310	1,035	2.87	4.51	1,030	9.14	14.43	220

CATALASE ACTIVITY

Measurements were made on the catalase activity of potato tissue from tubers that had been affected by the growth-inhibiting gas produced by apples and from tubers that had not been exposed to this gas. There is no universal agreement among plant physiologists concerning the significance of catalase activity in plants, but it is generally considered that its intensity is a measure of physiological activity and that it is associated with the function of respiration.

The measurements of catalase intensity were made from sprout and from tuber tissues according to the method adapted by Davis (*5*). It was found by preliminary tests that the catalase activity of sprouting potato tubers is greatest near the eyes, and tissue surrounding the eyes was consequently used for the tests. To weighed samples of macerated tissue was added 5 cc of hydrogen peroxide, and the amount of oxygen evolved in 5 minutes' time was measured by the displacement of water in a burette. The results obtained and the computed amount of oxygen evolved per 100 mg of tissue are recorded in table 2.

TABLE 2.—*Effect of the growth-inhibiting gas from apples upon catalase activity in the sprout tissue and the tuber tissue of the potato*

Tissue and date	Normal tissue [1]			Affected tissue [2]		
	Weight of sample	Oxygen liberated	Oxygen liberated per 100 mg	Weight of sample	Oxygen liberated	Oxygen liberated per 100 mg
Sprout: [3]	*Mg*	*Cc*	*Cc*	*Mg*	*Cc*	*Cc*
Nov. 9, 1932	200	29.0	14.50	180	39.0	21.67
	200	34.0	17.00	170	40.2	23.65
Nov. 11, 1932	230	36.1	15.69	200	38.8	19.40
	350	37.1	10.60	200	52.0	26.00
Jan. 6, 1934	484	23.5	4.86	407	37.7	9.27
	421	14.6	3.47	473	38.7	8.18
Jan. 15, 1934	399	35.5	8.90	422	53.8	12.75
	414	41.0	9.90	262	55.1	21.03
	679	56.4	8.36	539	63.1	11.70
Feb. 26, 1934	442	39.0	8.82	535	76.4	14.28
	812	76.8	9.46	522	94.5	18.10
Feb. 27, 1934	375	53.1	14.16	160	48.5	30.28
	427	72.8	17.05	205	53.3	26.00
Tuber: [4]						
Nov. 11, 1932	380	41.8	11.00	400	58.6	14.65
	300	32.3	10.77	330	60.1	18.21
Feb. 26, 1934	729	50.6	6.94	562	60.2	10.71
	686	72.0	10.50	580	90.1	15.53
Feb. 27, 1934	296	33.8	11.42	187	63.8	34.12
	345	52.7	15.28	249	62.3	25.02

[1] Tissues not affected by the growth-inhibiting gas.
[2] Tissues affected by the growth-inhibiting gas.
[3] Percentage increase of oxygen liberated by abnormal over normal, 79.88.
[4] Pecrentage increase of oxygen liberated by abnormal over normal, 65.27.

The tests on comparative catalase activity indicate that potato sprout and tuber tissues that have been affected by the growth-inhibiting gas from apples contain decidedly more of that enzyme than do the normal tissues. The catalase from abnormal sprout tissue liberated 79.88 percent more oxygen than did this enzyme from the normal sprouts, and the catalase from abnormal tuber tissue liberated 65.27 percent more oxygen than did that from the normal tubers. If is of interest to note that potatoes that had been affected with the growth-inhibiting gas from apples had both a higher respiration intensity and a greater catalase activity than did the normal potatoes.

OXIDASE ACTIVITY

Comparisons were made of the oxidase activity of potato sprouts that had developed in the presence and in the absence of the growth-inhibiting gas from apples. The measurements of oxidase activity were made according to the simplified method of Bunzel (*3*).

A higher oxidase activity was recorded from abnormal sprouts than from normal sprouts, as is indicated in table 3. In a test made in January 1934 the oxidase activity from the abnormal sprouts was 11 percent higher than that from the control sprouts, and in a test made 2 weeks later, the oxidase activity from affected tubers was 36 percent higher than that from the control tubers.

TABLE 3.—*Effect of the growth-inhibiting gas from apples upon oxidase activity in the sprout tissue and the tuber tissue of the potato*

Tissue	Oxygen absorbed by—	
	Normal tissue	Abnormal tissue
	Cc	*Cc*
Sprout	4.899	5.467
Tuber	4.437	6.035

CHEMICAL ANALYSES OF NORMAL AND AFFECTED SPROUT AND TUBER TISSUES

Chemical analyses were made of normal potato tubers and sprouts, and these analyses were compared with those of similar tissues from potatoes that had been held under the influence of the growth-inhibiting gas from apples. Irish Cobbler tubers were cut into longitudinal halves, and half of each tuber was placed in an enclosed chamber that contained Winesap apples. The tuber halves were covered with a layer of moist sphagnum to provide suitable conditions for growth. The other tuber halves were placed in the control chamber and were covered and held under similar environmental conditions except that no apples were present. The potatoes were allowed to grow approximately 1 month. The sprouts were then removed and dried at 65° to 70° C. after first being heated to between 90° and 100° C. for an hour to stop enzymatic action. Tubers from both lots were sliced and similarly heated and dried.

The analyses recorded in table 4 indicate the variations in composition that existed between the affected and normal sprout and tuber tissues. The total nitrogen content of the compared samples was quite similar. The dried normal and abnormal sprout tissues contained, respectively, 3.70 and 3.66 percent total nitrogen, while the total nitrogen content of the normal and abnormal tuber tissues was respectively 2.12 and 2.11 percent.

TABLE 4.—*Chemical analyses*[1] *of sprout and tuber tissues from normal potatoes and from potatoes affected by the growth-inhibiting gas from apples*

Tissue	Total nitrogen	Albuminoid nitrogen	Total sugars	Reducing sugars	Starch	Hemicellulose	Crude fiber	Ash	Water
Sprout:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Normal	3.70	1.86	15.40	13.46	16.79	5.38	11.65	8.60	91.24
Abnormal	3.66	2.61	20.66	17.41	16.67	6.92	7.06	7.00	89.09
Tuber:									
Normal	2.12	1.08	.64	0					79.75
Abnormal	2.11	1.03	3.20	0					78.11

[1] Water analyses on a fresh-weight basis; other analyses on a dry-weight basis.

The albuminoid-nitrogen content of the dried normal and abnormal tuber tissues was respectively 1.08 and 1.03 percent. A greater difference in albuminoid-nitrogen content existed between the affected and the normal sprouts. The normal sprouts contained 1.86 percent of albuminoid-nitrogen, which is 50.27 percent of the total nitrogen content, while the abnormal sprouts contained 2.61 percent of albuminoid nitrogen or 71.31 percent of the total nitrogen content. While the total nitrogen content of the abnormal sprouts was similar to that of the normal sprouts, these analyses show that it was largely in the form of the insoluble albuminoid nitrogen. The abnormal sprouts contained only 57 percent as much protein-free nitrogen as did the normal sprouts.

Total sugars and reducing sugars were more abundant in the abnormal than in the normal sprouts. The normal sprouts contained approximately one-fourth less both of total sugars and of reducing sugars than did the abnormal sprouts.

Tubers that had been exposed to the growth-inhibiting gas for a month contained five times as much total sugars as the untreated control tubers. This accounts for the relatively sweeter taste of the potatoes that were stored with apples. Neither the normal nor the treated potatoes contained reducing sugars.

The dry-weight starch content of the stunted and the normal sprouts was similar, and the hemicellulose content of these sprouts was also similar. No determinations were made of starch, hemicellulose, crude fiber, or ash in the tubers.

The crude-fiber content of the dried normal and abnormal sprouts was, respectively, 11.65 and 7.06 percent, or a decrease of 40 percent in crude fiber in the affected sprout tissue. Ash constituted 8.6 percent of the dry weight of the normal sprouts and 7.0 percent of the dry weight of the abnormal sprouts.

Water constituted 91.24 percent of the normal sprouts and 89.09 percent of the abnormal sprouts before they were dried. The moisture contained in the fresh normal and the treated tubers was, respectively, 79.75 and 78.11 percent.

Abnormal physiological activities, including enzymatic action and synthesis of carbohydrates and proteins, are induced by the growth-inhibiting gas and are apparently related to the morphological changes that occur in affected potato plants. The nature of this relationship has not been demonstrated, but it appears that the unbalance of carbohydrates and proteins within such plants is sufficiently great to affect their morphological development. Botjes (*1*) observed the premature formation of tubers from the eyes of potatoes from which repeated crops of sprouts had been removed and offered the hypothesis that this abnormal tuber formation is caused by an unbalanced carbohydrate and protein ratio in the mother tuber. He suggested that after repeated crops of sprouts have been removed, a shortage of proteins may finally result, under which conditions the formation of normal sprout tissue is hindered. A hypothesis may likewise be entertained that the gas from apple fruits may cause the permeability of the protoplasm of the potato to be changed, or that because of the effect of this gas on enzymatic action or on other functions, the potato may be unable to supply the developing sprout with the required proportion of carbohydrates and proteins. Analyses of normal and abnormal sprouts indicate that an unbalanced ratio of sugars

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and nitrogen compounds occurred in the abnormal sprouts. No great differences occurred in the amount of total nitrogenous materials in the compared sprouts, but albuminoid nitrogen was more abundant in the abnormal sprouts. The abnormal sprouts contained only about 57 percent as much protein-free nitrogen as did the normal ones. Accompanying the reduction in protein-free nitrogen, the abnormal sprouts contained an excess of 34 percent in total sugars and 29 percent in reducing sugars. The effect of this abnormal ratio of carbohydrates and protein-free nitrogen may very probably be the cause of a decided hindrance in normal sprout development.

INVESTIGATIONS TO IDENTIFY THE GROWTH-INHIBITING GAS FROM APPLES

Attempts were made in these investigations to identify the volatile growth-inhibiting substance that is produced by apples. The characteristics of this gas were studied from its reaction to certain solvents, absorbents, and oxidizers. Sprouting potato tubers were also treated with the gases of various chemical substances, and the effect of these chemicals upon sprout growth was noted.

A stream of air containing the volatile products from apples was treated with a solvent, absorbent, or oxidizing agent, and then drawn through a closed chamber that contained potatoes planted in moist soil. The index used to determine whether the growth-inhibiting gas had been destroyed was the ability of the potatoes to develop normal sprouts. The duration of the tests was approximately 14 days, which allowed time for sprout growth of the tubers.

The growth-inhibiting gas from apples was not removed or destroyed following dispersion through ethyl alcohol, mineral oil, petroleum ether, toluene, benzene, amylacetate, or potassium hydroxide. Abnormal sprouting of the potatoes occurred after the growth-inhibiting gas had been treated with these substances.

Air containing the gases from apples was passed through an electric furnace by way of a pyrex tube filled with a mixture of 1 part of platinized asbestos and 3 parts of black copper oxide. The treated air was then drawn through chambers that contained growing potatoes. The furnace was kept at a glowing-red temperature during the 14-day period of the test. Normal sprout growth resulted from the test potatoes, as is shown in figure 6, *B*, indicating that the growth-inhibiting gas was destroyed under the oxidizing conditions at this temperature.

Activated charcoal (Norit), which had been held at 100° C. for 36 hours before the test, was used in an attempt to adsorb the growth-inhibiting gas by slowly drawing the gases from apples through a 29-inch column of this adsorbent. No evidence was obtained that the growth-inhibiting gas was adsorbed, inasmuch as characteristically inhibited sprouts developed from the indicator potatoes.

Attempts were made to oxidize the growth-inhibiting gas from apples by dispersing it through a 2-percent solution of potassium permanganate made alkaline by the addition of 2 percent of potassium hydroxide. The resultant sprout growth of the tubers was not normal, but slowly elongating, somewhat thickened sprouts that were intermediate between normal and abnormal were produced in one of the tests. Evidently the growth-inhibiting gas was not completely oxidized.

Absorption of the growth-inhibiting gas from apples was attempted with sulphuric acid, fuming sulphuric acid, and chlorosulphonic acid. The gases from apples were passed through these acids in a Bowen potash-absorption bulb. Moisture was removed from the air stream with calcium chloride before the gases entered the absorption bulb, and the acid fumes were removed with a 40-percent potassium hydroxide solution before the air stream entered the potato-growing chambers. The growth-inhibiting gas was destroyed both by fuming

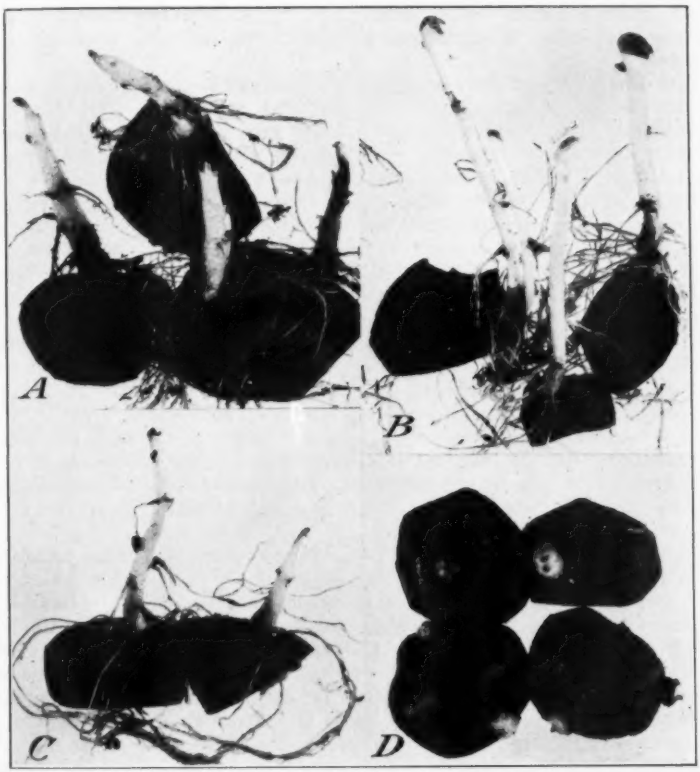

FIGURE 6.—The effect of absorption or oxidation on the growth-inhibiting gas from apples: *A*, Normal sprouts produced after the growth-inhibiting gas had been absorbed with chlorosulphonic acid; *B*, normal sprouts produced after the growth-inhibiting substance had been oxidized under high temperatures; *C*, control, normal sprouts from tubers not subjected to the growth-inhibiting substance; *D*, control, abnormal sprouts from tubers that were supplied with air containing the gases from apple fruits, the air stream being a portion of the supply used for *A* and *B*.

sulphuric acid and by chlorosulphonic acid,[4] as was indicated by the development of normal sprouts from the potatoes that served as indicators (fig. 6, *A*). Sulphuric acid (nonfuming) did not completely absorb the growth-inhibiting gas, and abnormal sprouts developed from the indicator potatoes.

An attempt was made to absorb the growth-inhibiting gas from apples with bromine. The gases from apples were dispersed through

[4] Reported as an absorbent by Botjes (*2*).

bromine-saturated water, then through a bromine-saturated atmosphere, after which the bromine vapors were stripped from the air stream by dispersion through potassium hydroxide. This absorbent destroyed the growth-inhibiting gas and allowed normal sprouts to develop from the tubers that served as indicators.

In the studies above described, the growth-inhibiting gas from apples was destroyed by substances that are known to react with ethylene. It was oxidized at a high temperature and apparently also by potassium permanganate. Treatments with fuming sulphuric acid, chlorosulphonic acid, and bromine (substances known to be efficient absorbents of ethylene) destroyed its growth-inhibiting property.

Certain effects on the potato plant of the gas from apples are similar to previously described effects of ethylene on other plants, and the possibility that ethylene is the casual growth-inhibiting substance from apples was entertained early in this investigation. Denny (*6*) found that ethylene caused green lemons to respire from 100 to 250 percent more carbon dioxide than normal, and Regeimbal et al. (*9*) noted that ethylene hastened the ripening of bananas, in which the liberation of carbon dioxide was doubled or trebled. It was found in the present investigations that approximately 100 percent more carbon dioxide than normal was respired by sprouting potatoes when they were exposed to the growth-inhibiting gas from apples.

Crocker (*4*) found that ethylene, acetylene, butylene, propylene, and carbon monoxide produce epinasty of the leaves of certain plants. In the present investigation this reaction was produced in potato plants both with ethylene and with the gas from apples.

Above-ground portions of the potato plant were confined in an atmosphere that contained 1 part of ethylene to 20,000 parts of air. The response of the leaves to this gas was similar to the response that followed when potato plants were held in closed containers with apple fruits.

Potato seed pieces planted in moistened sphagnum were confined in a similar concentration of ethylene in order to note the effect of this gas upon the development of sprouts and in order to compare their response with the effect that is produced by the growth-inhibiting gas from apples. The ethylene was renewed at 12-hour intervals after the air in the bell jars that enclosed the experimental potatoes had been completely changed. The effect of ethylene on the potato sprouts was not distinguishable from the effect that is produced by the growth-inhibiting gas from apples. The abnormal sprout growth that developed as a result of the treatment with ethylene is illustrated in figure 7.

Studies were made to identify the growth-inhibiting substance from apples. Fuming sulphuric acid, as was noted above, proved an efficient absorbent of this gas, and it was consequently employed in this investigation. The gases from apples were dispersed continuously for a period of approximately 3 weeks through fuming sulphuric acid in Bowen potash bulbs. The spent acid was made alkaline with sodium hydroxide, refluxed to hydrolyze any ester present, distilled, and the distillate, after being oxidized, tested with the aldehyde fuchsin reagent. A positive aldehyde test was obtained, which suggests that the acid reacted with an olefin, that the resulting ester was hydrolyzed to a primary alcohol, and that the primary alcohol

was oxidized to an aldehyde. This behavior indicates the probability that the growth-inhibiting gas is ethylene [5] and not acetylene, butylene, or propylene. The possibility that the growth-inhibiting gas from apples is acetylene is unlikely because acetaldehyde, formed when the acetylene-sulphuric acid addition product is hydrolyzed, is probably destroyed during the 3-hour period of refluxing in the presence of an alkali. The assumption is made that the growth-inhibiting gas is ethylene rather than any other olefin because the aldehyde

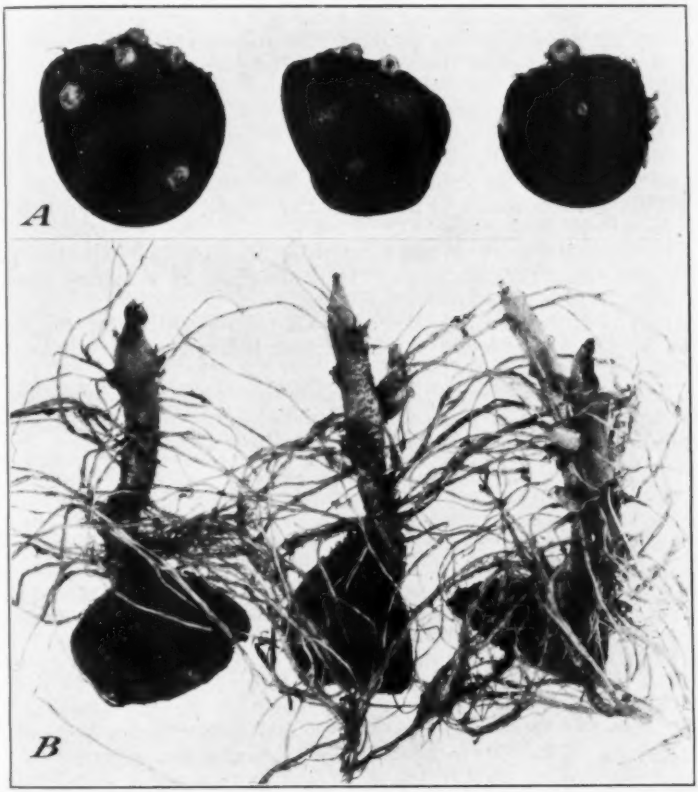

FIGURE 7.—Effect of ethylene on potato-sprout production: *A*, Sprout growth from tubers held with ethylene, 1 part to 20,000 parts of air; *B*, control tubers held under a similar environment except that no ethylene was present.

fuchsin test indicated the presence of a primary alcohol, while the homologues of ethylene tend to form secondary and tertiary alcohols after addition reactions and subsequent hydrolysis of resulting esters. Upon oxidation, secondary alcohols yield ketones, which react relatively slowly with the fuchsin reagent, while tertiary alcohols are broken up into ketones and acids.

[5] Gane (*8*) identified the growth-inhibiting gas from apples as ethylene by employing bromine as the absorbent. The investigations herein described were completed before Gane's report was noted, and they corroborate his conclusions.

SUMMARY

A volatile substance, normally produced by apple fruits, causes striking morphological and physiological abnormalities in the potato.

This volatile substance is produced by the mesocarp and endocarp of sound ripe apples. Pears and hawthorn fruits also emanate this gas. Affected potato sprouts produce an abnormal radial growth, while longitudinal growth practically ceases, and a tuberlike structure results. Epinasty occurs in affected leaves. Apical growth of the green plant ceases, but radial enlargement results in the younger portions of the stems and in the leaf petioles. Respiration and catalase activity are approximately doubled, the oxidase activity is increased, and a change occurs in the nitrogen-carbohydrate balance of sprouts and tubers.

The sprouting of potatoes in storage can be practically stopped and the tubers can be kept in a well-preserved condition by supplying a sufficient concentration of the growth-inhibiting gas. The total sugar content of the tubers is, however, increased, giving them a sweet flavor. Potatoes to be planted are apparently not injured by the growth-inhibiting gas.

The growth-inhibiting gas is oxidized at high temperatures and with potassium permanganate. It is absorbed by bromine, fuming sulphuric acid, and chlorosulphonic acid.

Analysis of the absorption products obtained by treating the gases from apples with fuming sulphuric acid indicates that ethylene is produced by these fruits.

The effect of ethylene upon sprout development and on the green portions of the potato plant was indistinguishable from the effect that is produced by the growth-inhibiting gas from apples.

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SEED GERMINATION IN LOBELIA, WITH SPECIAL REFERENCE TO THE INFLUENCE OF LIGHT ON LOBELIA INFLATA [1]

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INTRODUCTION

The Indian tobacco, *Lobelia inflata* L., a common native annual of eastern North America, is the source of alkaloids used in certain drugs. Most of these alkaloids are obtained from wild plants. Experimental plantings have been made, however, to determine the effect of various treatments on the alkaloidal content of *L. inflata* under cultivation,[3] and in recent years attempts have been made to grow it commercially.

Six years ago several samples of *Lobelia inflata* seed were submitted to the writer with the complaint that when planted the seed had failed to grow. Germination tests on these seeds had also been unsuccessful. The question then arose, Are these seeds immature, dormant, or dead? After subjecting lots of these seeds to various treatments employed to induce germination, such as alternating temperatures, chilling, freezing, soaking in sulphuric acid, hydrochloric acid, or hot water, and removal of seed coats, it was found that some of the samples were dead, and that strong light was necessary to obtain any germination from the others. The exact age of the seed samples was unknown, and no information was available concerning the condition of the seeds at the time of harvest. In order to obtain information on the effect of age upon the conditions necessary for the germination of *L. inflata* an experiment was begun in 1930 with fresh seeds of known age.

MATERIALS AND PROCEDURE

Seeds of *Lobelia inflata* were obtained from six localities: (1) Marion, Va.; (2) Asheville, N. C.; (3) Statesville, N. C.; (4) Chateaugay, N. Y.; (5) Newport, Vt.; and (6) Lenox, Mass. The first three lots were commercial seeds from the crop of 1929, supplied by J. T. Lloyd of Cincinnati, Ohio. The last three were harvested from wild plants when the fruits were ripe, about the middle of September 1930. These seeds were allowed to air-dry in the laboratory and were stored in a dark case in tightly stoppered glass bottles until they were used for making germination tests.

All tests were made in duplicate with lots of 100 seeds. Preliminary germination tests were made with soil, water, paper towels, and unburned clay flower pots as substrata. The best germination was obtained in water or by placing the seeds on the moistened surface of a clay flowerpot. Small 1-inch pots were used for the germination

[1] Received for publication Jan. 11, 1936; issued May 1936.

[2] The writer is indebted to S. R. Patrick and Helen Hazard for assistance in making the germination tests in 1934 and 1935.

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tests. The clay pots were inverted in a glass bowl of water so that the water reached within 5 mm of the surface holding the seeds. The bowls were covered with Petri dishes so as to provide a moist, well-lighted germination chamber. One series of chambers was set in a well-lighted room near a south window; the other series was placed in a dark chamber in the same laboratory room.

The germinators were examined daily and the germinated seedlings were counted and removed on the ninth to twelfth day and at approximately 10-day intervals thereafter for 40 to 60 days. In the earlier tests the germinators in the dark were opened only in a dark room with artificial light, but later, when it was found that the short exposure to daylight did not affect the germination of the soaked seeds, the seeds were examined from time to time in daylight. Germination tests were made of six samples over a period of from 4 to 6 years.

Germination tests of several other species of *Lobelia* were made, by the methods already described, in order to compare the results with those obtained with *L. inflata*. The following lots of seed were harvested in the autumn of 1930 from mature fruits from plants growing wild in various parts of New York: *L. cardinalis* L., *L. dortmanna* L., *L. kalmii* L., and *L. siphilitica* L. In addition, seed of *L. tenuior* R. Br. and five varieties of *L. erinus* L. were obtained from a commercial seed dealer.

DATA AND DISCUSSION

The results of germination tests with *Lobelia inflata* seeds from 1 to 6 years of age are recorded in table 1.

TABLE 1.—*The germination of Lobelia inflata seeds in light and darkness*

[100 seeds were used in each test]

Lot no., source of sample, and year of harvest	Year of test	Duration of germination test		Germination in light [1]
		Begun	Ended	
				Percent
1, Marion, Va., 1929.	1930	Apr. 17	July 15	16
	1930	May 1	...do.....	59
	1930	May 12	...do.....	55
	1931	Apr. 12	Apr. 27	67
	1933	Mar. 16	Apr. 20	40
	1934	Mar. 1	Mar. 31	48
	1935	Feb. 10	Apr. 8	28
2, Asheville, N. C., 1929.	1930	Apr. 17	July 15	48
	1930	May 1	...do.....	46
	1930	May 12	...do.....	29
	1931	Apr. 12	Apr. 27	55
	1933	Mar. 16	Apr. 20	42
	1934	Mar. 1	Mar. 31	41
	1935	Feb. 10	Apr. 8	34
3, Statesville, N. C., 1929.	1930	Apr. 17	July 15	64
	1930	May 1	...do.....	88
3, Statesville, N. C., 1929—Continued.	1930	May 12	July 15	80
	1931	Apr. 12	Apr. 27	81
	1933	Mar. 16	Apr. 20	44
	1934	Mar. 1	Mar. 31	36
	1935	Feb. 10	Apr. 8	40
4, Chateaugay, N. Y., 1930.	1931	Jan. 2	Feb. 18	0
	1933	Mar. 16	Apr. 20	28
	1934	Mar. 1	Mar. 31	4
	1935	Feb. 10	Apr. 8	20
5, Newport, Vt., 1930.	1931	Jan. 2	Feb. 18	0
	1933	Mar. 16	Apr. 20	33
	1934	Apr. 4	May 4	93
	1935	Mar. 29	Apr. 28	94
6, Lenox, Mass., 1930.	1931	Jan. 2	Feb. 18	0
	1933	Mar. 16	Apr. 20	3
	1934	Apr. 4	May 4	24

[1] There was no germination in darkness but when the seeds were exposed to light at the end of the 1935 germination test in darkness the following percentages of germination were obtained for the lot numbers indicated: 1, 36; 2, 23; 3, 68; 5, 97.

All of the samples gave some germination in sunlight. No seeds germinated before the eighth day. Germination usually began on the ninth day, and approximately 80 percent of the total germination occurred between the ninth and the twentieth days. There was

considerable variation in the percentage of germination in the samples of seed from different sources. All six samples continued to remain viable for from 4 to 6 years, or the duration of the experiment. The figures for samples 4, 5, and 6 indicate a period of low germination during the first year, possibly a period of dormancy due to other factors than light. In general, the percentage of germination increased during the second to fourth year and then decreased somewhat.

At no time did even a single seed germinate in the germinators kept in darkness. Some of the lots of seed after having been kept in the dark for 30 to 55 days without germinating were transferred to a room where they were exposed to sunlight, and within 9 to 30 days from 23 to 94 percent germination was obtained (table 1). These germination percentages were very similar to those obtained by placing similar samples of seed directly in the light. These results demonstrate the necessity of light for the germination of seeds of *Lobelia inflata*.

It was thought that if large lots of seeds were placed in germinators, possibly a few seeds would be found to germinate in darkness. Accordingly, lots of several thousand seeds were scattered on the surface of water cultures and placed in dark chambers, and similar control cultures were placed in sunlight. The cultures in the light germinated well, but not a single seed germinated among those kept in the dark.

To determine the influence of light on the germination of *Lobelia inflata* under field conditions, seeds of lots 1, 2, 3, and 5 were sown in rows in a garden (Dunkirk silty clay loam) on May 24, 1934. In one series the seeds were dropped on the surface of the soil and in another they were covered with about 1 cm of finely pulverized soil mixed with sand. No seedlings were visible before June 10, but from then on numerous plants began to appear from the surface-sown seed. Only three plants were found in the rows in which the seed had been covered. It would appear that in growing *L. inflata* from seed good results may be expected by sowing the seed on the surface of a finely prepared seedbed, as is frequently done with tobacco seed.[4] Stockberger,[5] in discussing the planting of *L. inflata* seed states that it is better not to cover them but to sow them on the surface of the soil.

The striking results obtained with *Lobelia inflata* suggested the desirability of testing other species of *Lobelia* to determine whether they would respond to light in the same manner. The results of these tests, recorded in table 2, show that *L. cardinalis* and *L. siphilitica*, like *L. inflata*, failed to germinate in darkness.

The results of the tests with *L. dortmanna* and *L. kalmii* are inconclusive. In *L. dortmanna*, the water lobelia, an aquatic plant of shallow acid ponds and lakes, the seeds failed to germinate, probably because they had been killed by drying. These seeds normally mature in the water, or the capsules, borne on the slightly emersed peduncles, are but little raised above the surface of the water, so that they are kept continually moist until the seeds fall into the water. Seeds produced under such conditions probably do not withstand drying. To test the validity of this explanation, fresh capsules of

[4] KINCAID, R. H. EFFECTS OF CERTAIN ENVIRONMENTAL FACTORS ON GERMINATION OF FLORIDA CIGAR-WRAPPER TOBACCO SEEDS. Fla. Agr. Expt. Sta. Bull. 277, 47 pp., illus. 1935.

[5] STOCKBERGER, W. W. DRUG PLANTS UNDER CULTIVATION. U. S. Dept. Agr. Farmers' Bull. 636. 1920.

L. dortmanna were harvested in Round Lake, Sullivan County, N. Y., on September 5, 1935. The seeds were placed in cold storage at 1° to 3° C. and kept there from September 15 to November 10, 1935. One lot was stored in jars of lake water from the time of harvest until the germination tests and another lot was air-dried and stored in a manila envelope at room temperature. Duplicate samples of 100 seeds from each lot were placed in glass jars covered with 6 cm of water. One set was placed in sunlight and the other was placed in a dark chamber. At the end of 15 days, in the seeds that had never been allowed to dry, 73 percent of those in the light and 57 percent of those in the dark had germinated. On the other hand, none of the dried seeds germinated, even after a subsequent storage of 4 weeks in water at 1° to 3° C. These results show that seeds of *L. dortmanna* do not require light for germination, and also that they lose their viability upon drying.

TABLE 2.—*The germination of Lobelia seeds of various species in light and darkness*

[100 seeds were used in each test]

Lot no., source of sample, and year of harvest [1]	Species	Year of test	Duration of germination test		Germination	
			Begun	Ended	In darkness	In light
					Percent	*Percent*
7, Ithaca, N. Y., 1930	*L. cardinalis*	1931	Jan. 2	May 5	0	0
		1932	Apr. 12	May 5	0	13
		1934	Apr. 4	May 4	0	94
		1935	Feb. 21	Apr. 8	0	54
8, Meacham Lake, N. Y., 1930.	*L. dortmanna*	1930–31	Sept. 23, 1930	Apr. 10, 1931	0	0
		1932	Apr. 12	May 5	0	0
		1934	Apr. 4	May 4	0	0
		1935	Feb. 21	Apr. 8	0	0
9, Osgood Pond, N. Y., 1930	do	1930–31	Sept. 23, 1930	Apr. 10, 1931	0	0
		1932	Apr. 12	May 5	0	0
		1934	Apr. 4	May 4	0	0
10, Massena, N. Y., 1930	*L. kalmii*	1930–31	Sept. 23, 1930	Apr. 10, 1931	0	0
		1932	Apr. 12	May 5	0	0
		1934	Apr. 4	May 4	0	0
		1935	Feb. 21	Apr. 8	0	0
11, Whitehouse Creek, N. Y., 1930	do	1930–31	Sept. 23, 1930	Apr. 10, 1931	2	8
		1932	Apr. 12	May 5	0	0
		1934	Apr. 4	May 4	0	0
12, Ithaca, N. Y., 1930	*L. siphilitica*	1931	Jan. 2	Apr. 10	0	0
		1932	Apr. 12	May 5	0	0
		1934	Apr. 4	May 4	0	83
		1935	Feb. 21	Apr. 8	0	36
13, Ithaca, N. Y., 1930	do	1931	Jan. 2	Apr. 10	0	0
		1932	Apr. 12	May 5	0	0
		1934	Apr. 4	May 4	0	51
14, commercial	*L. tenuior*	1931	Jan. 2	Feb. 10	80	88
		1932	Apr. 12	Apr. 27	87	92
15, commercial	*L. erinus* var. gracilis.	1931	Jan. 2	Feb. 10	82	86
		1932	Apr. 12	Apr. 27	81	84
16, commercial	*L. erinus*, White Gem.	1931	Jan. 2	Feb. 10	79	76
		1932	Apr. 12	Apr. 27	85	82
17, commercial	*L. erinus*, Blue Emperor.	1931	Jan. 2	Feb. 10	83	88
		1932	Apr. 12	Apr. 27	81	96
18, commercial	*L. erinus*, Crystal Palace.	1931	Jan. 2	Feb. 10	71	76
		1932	Apr. 12	Apr. 27	66	73
19, commercial	*L. erinus* var. speciosa.	1931	Jan. 2	Feb. 10	93	95
		1932	Apr. 12	Apr. 27	95	94

[1] The seed of lots 14–19 were probably of the 1930 harvest.

The poor germination obtained in *Lobelia kalmii* might possibly have been due to the presence of dead seed. However, the small percentage of germination during the first year and the absence of

germination in subsequent years (table 2) suggest that drying of the seed may have been the cause. The habitat of *L. kalmii*—wet bogs, springy banks, and dripping wet cliffs—would naturally supply a moist stratum upon which the mature seeds fall as they are shed from the plant. To verify the effect of drying upon its seeds would require germination tests with fresh undried seeds.

No germination was obtained from the seed of *L. cardinalis* and *L. siphilitica* in the tests made about 1 year after the seed was harvested; the best germination was obtained 4 years after harvest. This behavior suggests a secondary dormancy of the seed brought about by the dry conditions under which the seeds were stored.

The six forms of cultivated lobelias, *Lobelia erinus* and *L. tenuior*, all gave good to very good percentages of germination with no significant differences between tests conducted in light and darkness.

A number of plants in widely unrelated families have been shown to have seeds that are light-sensitive or whose germination is favored by light. Several explanations of the direct or indirect action of light on such seeds have been discussed by Gardner,[6] Kommerell,[7] Kinzel,[8] Kincaid,[9] and others. In all the examples found in the literature in which seed germination was favored by light, some seeds also germinated in darkness. In the present investigation a good percentage of seeds of *Lobelia inflata* germinated in the light, but out of upwards of 500,000 seeds placed in germinators in the dark, not one germinated unless subsequently exposed to sunlight.

SUMMARY

It has been demonstrated that light is necessary for the germination of *Lobelia inflata* seeds. Of the several other treatments given to the seeds, none acted as a substitute for light to bring about germination. When *L. inflata* seeds were sown on the soil surface in a garden, good germination was obtained, but practically no germination was obtained when the seeds were covered with 1 cm of soil. The seeds retained their viability for at least 5 years in dry storage under laboratory conditions.

Tests with other species of *Lobelia* indicate that *L. cardinalis* and *L. siphilitica* also require light for germination.

The seeds of *L. tenuior*, five forms of *L. erinus*, and *L. dortmanna* germinate about equally well in light or darkness.

Seeds of *Lobelia dortmanna* lose their viability upon drying. Seeds stored in water at a temperature of 1° to 3° C. retain their viability for at least 4 months.

[6] GARDNER, W. A. EFFECT OF LIGHT ON THE GERMINATION OF LIGHT-SENSITIVE SEEDS. Bot. Gaz. 71: 249–288. 1921.

[7] KOMMERELL, E. QUANTITIVE VERSUCHE ÜBER DEN EINFLUSS DES LICHTES VERSCHIEDENER WELLENLÄNGEN AUF DIE KEIMUNG DER SAMEN. Jahrb. Wiss. Bot. 66: 461–512, illus. 1926.

[8] KINZEL, W. GRENZEN DER FÖRDERLICHEN EINWIRKUNG VON FROST UND LICHT BEI DER SAMENKEIMUNG. Angew. Bot. 12: 16–22. 1930.

[9] KINCAID, R. R. See footnote 4.

TIME INTERVAL BETWEEN EGGS OF RHODE ISLAND RED PULLETS[1]

By F. A. Hays

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INTRODUCTION

The time interval between eggs laid on successive days has been studied by a number of investigators. Warren and Scott[2] have recently reported the results of carefully controlled experiments to obtain more definite information relative to the time element in egg formation. They report for White Leghorns that the average time between eggs laid on successive days is 25.1 hours. Their studies indicate also that the variation between individual birds in time interval between eggs is due largely to variations in the time the egg is held in the uterus.

The studies reported here on Rhode Island Red pullets in the first laying year were undertaken to discover possible relationships between the time between eggs within the clutch of individuals and some important fecundity and reproductive characters.

DATA RECORDED

Four hundred and nine pedigreed Rhode Island Red pullets bred for high fecundity and hatched from March 8 to May 22, 1926, were trap-nested hourly for a full laying year and the time of laying was recorded on the hour. In other words, eggs taken from the trapnests from 9 to 10 o'clock were marked as laid at 9 o'clock, and so on throughout the day. Such a method of recording time was reasonably accurate within 1 hour but would in general tend somewhat to increase the recorded time interval over the actual time interval. The mean time interval within clutches from first pullet egg to March 1 was calculated for each individual, together with winter clutch size, winter egg production, hatching season egg weight, hatchability, persistency, and annual egg production. Monthly time between eggs was also calculated for 119 of these birds that were in one house, together with the monthly egg record of each individual.

CHANGES IN TIME INTERVAL BETWEEN EGGS DURING THE PULLET LAYING YEAR

Figure 1, based on the records of 119 birds, shows clearly that the mean time between eggs laid on successive days varies greatly throughout the first laying year. The great majority of the birds used in this study began their laying year in October. Figure 1 shows that pullets tend to begin their year with a relatively short period between

[1] Received for publication Dec. 9, 1935; issued May 1936. Contribution no. 232 of the Massachusetts Agricultural Experiment Station.

[2] Warren, D. C., and Scott, H. M. The time factor in egg formation. Poultry Sci. 14: 195-207, illus. 1935.

eggs. The mean time interval for October was 26 hours. As the season advances into winter there is a rapid and consistent increase in time between successive eggs through the month of February. The records for March show a pronounced shortening of the time interval, and April is characterized by the shortest time interval of the year, 25.7 hours. As spring advances into summer the time between eggs of a clutch increases and remains at a medium level from June through September. Examination of the individual records shows that the shortest monthly time interval recorded was 23 hours for one bird during June and 23.4 hours for the same individual during April. Several birds showed a monthly mean of 24 hours for April. The maximum time interval recorded was 31.7 hours for one bird during February. The mean time interval for the entire year was 26.49 hours.

The mean monthly egg production of the same group of birds is shown in figure 1. For the winter season up to March 1 there is a

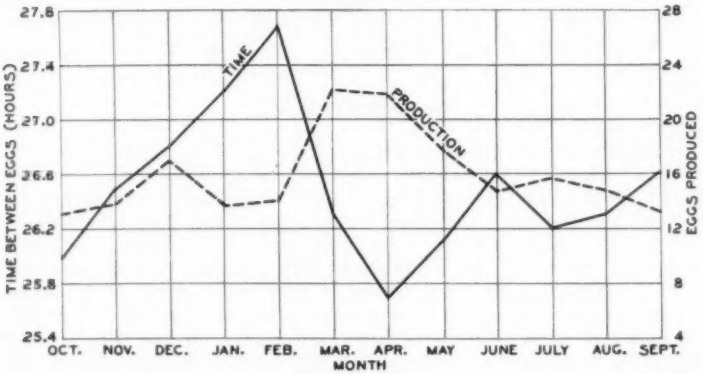

FIGURE 1.—Relation between monthly egg production and monthly interval between eggs, based on the records of 119 birds.

tendency for monthly egg production to decrease as the time between eggs becomes greater. The spring season of normally heavy laying during March, April, and May is accompanied by a shortened interval between eggs. The mean egg production during March was 22.34 and during April it was 21.97. During June, July, and August egg production approaches the mean for the year, which is 16.15 eggs, and the interval between eggs is close to the yearly mean of 26.49 hours.

In general, the data in figure 1 show that heavy egg production is associated with a short interval between successive eggs in a manner similar to the association between large clutch size and high egg production pointed out by Hays and Sanborn.[3]

INTERVAL BETWEEN EGGS IN WINTER AS RELATED TO SIZE OF WINTER CLUTCH

Data are available on 409 individuals representing a normal sample for time interval, as figure 2 indicates. The χ^2 test gave a

[3] HAYS, F. A., and SANBORN, R. TYPES OF INTENSITY IN RHODE ISLAND REDS. Mass. Agr. Expt. Sta. Bull. 286, 11 pp. 1932.

value for P of 0.3688. Table 1 presents the mean clutch size of the different time-interval classes for 394 birds. The data show a constant decline in clutch size as the time between eggs increases up to about 28.5 hours. When the time is greater than 28.5 hours, clutch size shows no orderly decrease.

TABLE 1.—*Time between eggs as related to winter clutch size and winter production in 394 birds*

Time between eggs during winter (hours)	Birds	Egg clutch size in winter	Winter egg production	Time between eggs during winter (hours)	Birds	Egg clutch size in winter	Winter egg production	Time between eggs during winter (hours)	Birds	Egg clutch size in winter	Winter egg production
	Number	*Number*	*Number*		*Number*	*Number*	*Number*		*Number*	*Number*	*Number*
24.0---	1	10.50	65.50	26.5---	64	2.77	66.59	29.0---	17	1.44	32.56
24.5---	17	6.00	85.50	27.0---	53	2.25	60.03	29.5---	5	1.60	35.50
25.0---	23	5.00	87.24	27.5---	47	2.06	54.86	30.0---	4	1.13	10.50
25.5---	37	3.64	74.42	28.0---	51	1.85	49.42	30.5---	1	1.50	15.50
26.0---	50	3.15	76.90	28.5---	23	1.63	41.15	31.0---	1	1.00	25.50

Figure 3 presents the same data in graphic form. The rate of decrease in clutch size with increased time intervals does not follow a straight line, but rather a hyperbolic curve. The equation of the

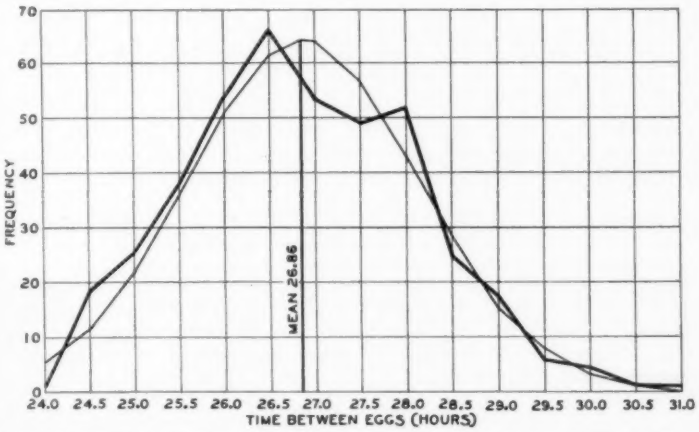

FIGURE 2.—Frequency distribution of time between eggs during winter (to Mar. 1, 1926) for 409 birds.

curve shown in the figure is $y^2-0.52xy+96.67y+5.82x^2-326.19x=-4{,}656.01$. This curve fits the actual data very closely for the clutch-size classes that correspond with time-interval classes up to 28.5 hours. This includes 366 out of a total of 394 birds.

These data furnish evidence that large clutch size definitely depends on short time intervals between eggs and that clutch size does actually measure the rate of functioning of the female reproductive system. The mean winter time interval was 26.9 hours.

INTERVAL BETWEEN EGGS IN WINTER AS RELATED TO WINTER EGG PRODUCTION

In table 1 is shown the mean winter egg production for the different classes of layers with respect to time between eggs. There is a generally consistent and regular decline in winter egg production as the time between eggs increases. With an increase in time from 24.5 hours to 29.5 hours there was a decrease in winter egg production from 85.5 eggs to 35.5 eggs. These data indicate that any increase in

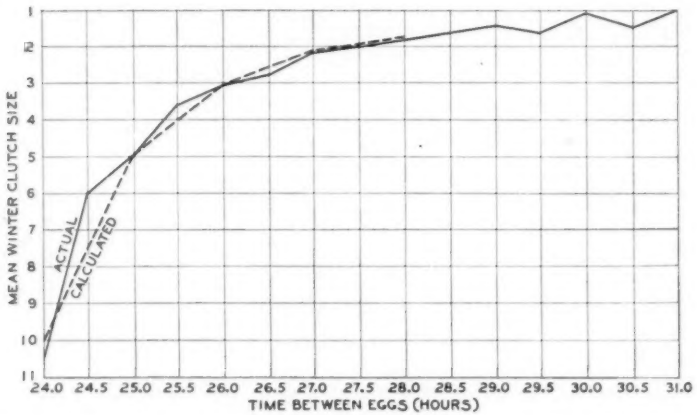

FIGURE 3.—Actual and calculated time between eggs during winter, as related to mean size of clutch, for 394 birds.

the time interval between eggs in a clutch is likely to be followed by a significant decline in egg production.

INTERVAL BETWEEN EGGS IN WINTER AS RELATED TO HATCHING-SEASON EGG WEIGHT

A group of 47 of these birds was used for breeding in the spring of 1927. The eggs were weighed during the last week of February and the first week of March to secure an approximation of the egg weight of each bird during the hatching season extending from February 22 to March 20.

TABLE 2.—*Time between eggs as related to egg weight during hatching season and to hatchability in 47 birds used for breeding*

Time between eggs during winter (hours)	Birds	Egg weight during hatching season	Hatchability of eggs	Time between eggs during winter (hours)	Birds	Egg weight during hatching season	Hatchability of eggs
	Number	*Grams*	*Percent*		*Number*	*Grams*	*Percent*
24.5	3	50.3	35.0	26.5	11	54.0	58.0
25.0	8	52.9	63.8	27.0	2	52.0	50.0
25.5	11	55.2	50.5	27.5	2	52.5	35.0
26.0	10	54.8	52.3				

In table 2 the mean egg weight of the birds classified with respect to time interval is presented. As the table shows, no birds were used for breeding that had a time interval greater than 27.5 hours; all were, therefore, intense layers. The maximum egg weight of 55.2 g occurred in the group with an average interval of 25.5 hours. When the interval was less than 25.5, or more than 26.5 hours, egg size was significantly below average. These limited data confirm the idea that very rapid laying is likely to be associated with small eggs, as Hays [4] has pointed out.

INTERVAL BETWEEN EGGS IN WINTER AS RELATED TO HATCHABILITY, PERSISTENCY, AND ANNUAL PRODUCTION

The relation between the mean time between eggs up to March 1 and percentage of fertile eggs that hatched is set forth in table 2. There is some evidence that the eggs of birds that lay successive eggs at 24.5- or 27.5-hour intervals may be low in hatchability. This is in agreement with the findings of Hays and Nicolaides [5] that birds laying eggs developed only to the pregastrula or early gastrula stage are likely to have low records of hatchability. The data further tend to show that when the eggs are laid at intervals greater than 27 hours the hatchability may be low. The most satisfactory hatchability was obtained from individuals having a mean time interval from 25 to 26.5 hours.

Persistency records covering the 365-day laying year are available for 246 birds. In table 3 the mean persistency record for the different winter season time-interval classes is recorded. The data show a consistent decrease in persistency with each increase in winter time interval up to about 27.5 hours, after which the decrease is less orderly. The data point to an important association between rapid rate of laying in winter and persistent laying at the end of the year. Such a relation is of considerable economic importance.

TABLE 3.—*Time between eggs as related to annual persistency and annual egg production in 246 birds*

Time between eggs during winter (hours)	Birds	Annual persistency	Annual egg production	Time between eggs during winter (hours)	Birds	Annual persistency	Annual egg production
	Number	*Days*	*Number*		*Number*	*Days*	*Number*
24.5	13	352.38	238.92	27.5	27	330.52	186.07
25.0	18	337.00	228.67	28.0	29	330.10	205.10
25.5	25	335.00	216.00	28.5	14	317.36	165.57
26.0	32	330.75	215.91	29.0	7	287.00	169.14
26.5	43	328.28	209.09	29.5	3	303.67	162.00
27.0	35	325.57	194.14				

The relation between time interval between eggs for the winter season and annual egg production is also shown in table 3. There is a generally regular decline in egg production with each increase in interval between eggs. In other words, the ability to manufacture eggs in rapid succession during the winter season is a very good indi-

[4] HAYS, F. A. INCREASE IN EGG WEIGHT DURING THE PULLET LAYING YEAR. Poultry Sci. Assoc. Proc. (1930) 22: 16–19, illus. 1931.

[5] HAYS, F. A., and NICOLAIDES, C. VARIABILITY IN DEVELOPMENT OF FRESH-LAID HEN EGGS. Poultry Sci. 13:74–80, illus. 1934.

cation that the bird will have a high annual egg record. The data show that an increase in time interval between eggs from 24.5 to 28.5 hours gave a decline in annual egg record from 239 eggs to 166 eggs. As the time interval becomes greater than the population mean of 26.9 hours there is a slight falling off in the rate of decrease in egg production in relation to increase in time interval.

SUMMARY

The mean time interval between eggs laid on successive days was calculated on 394 Rhode Island Reds from first pullet egg up to March 1. Monthly time intervals were determined on 119 of these birds which were housed together. A study was made of the relation of the time interval between successive eggs to several fecundity and reproductive characters. A number of significant relationships were observed, as follows:

(1) Monthly time interval between eggs reached the lowest level during April and stood at a relatively low level during the months of maximum egg production. The greatest time interval occurred during months of lowest production.

(2) Short time intervals between eggs for the winter season were characteristic for birds showing large clutch size.

(3) Short time intervals between eggs up to March 1 were characteristic of heavy winter producers.

(4) A mean winter season time interval of 25.5 hours or more was associated with maximum egg weight during the hatching season. When the time interval fell below 25.5 hours or was above 26.5 hours, there was a decrease in egg size.

(5) There was some evidence that a winter time interval as low as 24.5 hours may be associated with low hatchability.

(6) In the birds studied each increase in winter time interval tended to be followed by decreased persistency.

(7) Short winter time interval between eggs was definitely associated with high annual egg production. The winter time interval appears to have definite value in predicting what the egg record may be at the close of the pullet laying year.

ACCURACY OF THE DETERMINATION OF LEAD AND ARSENIC ON APPLES[1]

By DONALD E. H. FREAR and W. S. HODGKISS, *Department of Agricultural and Biological Chemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

The accuracy of the Gutzeit method for the determination of small amounts of arsenic has been studied by serveral workers (*2*, *3*, *4*, *8*),[2] but the accuracy of the determination of similar small amounts of lead has apparently not been investigated. Federal and State regulatory bodies have now concerned themselves with the amount of lead present on edible fruit as spray residue and have set up limits which legally may not be exceeded.

The legal limits for lead and arsenic trioxide during the season of 1935 have been set at 0.018 and 0.010 grain per pound, respectively. In view of these limits, and the fact that the limits for lead are being gradually reduced, the accuracy of the determinations of these elements becomes important to the chemist, for the analytical results must be interpreted in relation to these legal tolerances.

Any study of the accuracy of these determinations naturally divides itself into two parts: The accuracy of the sampling, and the accuracy of the determination itself. The summation of the errors in these two phases of the work represent the errors in the final determination.

Lead, when present on the surface of apples as spray residue, may combine chemically with the waxy coating of the fruit (*5*), and thus it presents a different problem from arsenic, which apparently does not so combine. In this study the accuracy of the chemical determination of lead will be discussed first, followed by a discussion of the accuracy of the estimation as a whole (including sampling) of both lead and arsenic. The accuracy of the chemical determination of arsenic by the Gutzeit method has been discussed at length (*4*, *7*), and will not be considered here.

ACCURACY OF THE CHEMICAL DETERMINATION OF LEAD BY THE PHOTOELECTRIC METHOD

The photoelectric method for the determination of lead on fruit, proposed by Frear and Haley (*6*), is briefly described in order that its advantages and limitations may be better understood.

Light from an electric bulb, regulated by a suitable rheostat, is allowed to shine upon the surface of a photronic cell (Weston no. 594), which is connected directly to a microammeter having a capacity of 200 microamperes. Between the source of light and the photronic cell is interposed a cylindrical glass tube containing the solution to be analyzed for lead. The reading of the microammeter is brought to the maximum of the instrument through an adjustment of the

[1] Received for publication Nov. 13, 1935; issued May 1936. Technical Paper No. 647 of the Pennsylvania Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 644.

intensity of the light by means of the rheostat. To the solution in the tube is then added sufficient sodium sulphide to precipitate completely the lead present as colloidal lead sulphide. The brown colloidal precipitate reduces the amount of light passing through the solution and falling upon the surface of the photronic cell. The relation between the amount of light transmitted, as measured by the microammeter connected with the cell, and the amount of lead present may be easily determined by standardization with known amounts of lead.

It is apparent that two sources of error are inherent in the method: The sum of the errors due to variation in technique, reagents, etc., which may be called the error of operation; and the error of standardization.

To determine the probable error of a single or of the mean of duplicate determinations at a level which will fall within the range of practical work, 50 determinations were made on solutions each containing 0.0004 g of lead as lead acetate (table 1). These solutions were each carried through the entire analytical procedure, including the digestion with concentrated sulphuric and nitric acids, which is used on samples of apple wash to destroy the organic material. The quantity of lead was selected at 0.0004 g since this is equivalent to a concentration of 0.020 grain of lead per pound of apples when an aliquot of the apple wash representing 140 g of apples is taken for analysis.

TABLE 1.—*Analyses of 50 aliquot portions of solution, each of which contained 0.4000 mg of lead*

Sample no.	Microammeter reading	Milligrams of lead	Sample no.	Microammeter reading	Milligrams of lead	Sample no.	Microammeter reading	Milligrams of lead
1	54	0.400	18	56	0.393	35	53	0.404
2	52	.410	19	53	.404	36	55	.396
3	52	.410	20	54	.400	37	54	.400
4	54	.400	21	52	.410	38	54	.400
5	55	.396	22	56	.393	39	54	.400
6	56	.393	23	57	.390	40	55	.396
7	53	.404	24	53	.404	41	54	.400
8	53	.404	25	53	.404	42	53	.404
9	53	.404	26	54	.400	43	55	.396
10	54	.400	27	52	.410	44	54	.400
11	55	.396	28	56	.393	45	56	.393
12	55	.396	29	56	.393	46	53	.404
13	54	.400	30	53	.404	47	52	.410
14	55	.396	31	52	.410	48	55	.396
15	56	.393	32	55	.396	49	54	.400
16	55	.396	33	54	.400	50	53	.404
17	56	.393	34	55	.396			

By the use of the formula $P.\ E.=\frac{0.6745\sigma}{\sqrt{n}}$, in which the standard deviation is calculated by the formula $\Sigma=\sqrt{\frac{D^2}{n}}$, the probable error of the microammeter readings obtained by analyzing the 50 samples was calculated. The mean value was 54 ± 0.13 microamperes, with a probable error of a single determination of ±0.89 microampere, and of the mean of duplicate determinations of ±0.63 microampere. Since with the instrument used it was not possible to make readings more closely than 1 microampere, the probable error of even a single determination was less than a measurable value.

To translate the microammeter readings into terms of lead concentration, a standardization curve was used which had been constructed by using the mean of 10 determinations at 5 different concentrations. The microammeter reading on this curve corresponding to 0.0004 g of lead was 54 microamperes. By the same formulae as above, the calculated probable error of a single determination was ± 0.0036 mg; and that of the mean of duplicate determinations was ± 0.0026 mg.

The probable error of the determination as a whole is further affected, by definition, by any error in the preparation of the curve of standardization. To include this factor, the formula $P.\ E. = \sqrt{(P.\ E._s)^2 + (P.\ E._{10})^2}$, in which $P.\ E.$ is the probable error of the method as a whole, $P.\ E._s$ is the probable error of a single determination, and $P.\ E._{10}$ is the probable error of the 10 determinations made to establish the point of reference on the curve of standardization. When the calculation is carried out, the true probable error of a single determination is ± 0.0038 mg. When the probable error of duplicate determinations is calculated, the value is ± 0.0028 mg.

A comparison of the relative accuracy of the lead method with that of the modified Gutzeit method for the determination of arsenic studied by Barnes and Murray (*4*) indicates that when the quantities determined are considered the method for lead exhibits a relatively higher accuracy than the method for arsenic. Barnes and Murray state that with a solution containing 0.0273 mg of As_2O_3, the probable error of duplicate determinations was ± 0.0023 mg and, when the probable error of standardization was considered, the true probable error of the determination was ± 0.0025 mg, the mean of 10 individuals being used to locate each point on the standardization curve. The corresponding figures found for the lead determination, in which the standard solution contained 0.4000 mg, calculated in the same manner, were respectively ± 0.0026 mg and ± 0.0028 mg.

ACCURACY OF THE FINAL RESULTS FROM THE ANALYSIS OF LEAD AND ARSENIC

To include the errors due to sampling, which obviously affect the accuracy of the final values for lead and arsenic, the procedure described below was followed.

Samples of apples, ranging in weight from approximately 7 to 20 kg, were selected, usually picked from the lower limbs of trees in the orchards. Ordinary precautions were taken in handling, and apples of nearly the same size were selected. These samples were taken to the laboratory, and from them two lots of 700 g each were taken, and each washed thoroughly with boiling 1.5-percent hydrochloric acid with 1.0 percent of sodium chloride added. The washings, when cool, were made up to 500 ml with acid of the same concentration. The lead was determined on an aliquot by the method described above (*6*), and arsenic was determined by the Gutzeit method as described in the Official Methods (*1*). In all, 164 lots were analyzed, with a wide range of lead and arsenic content. The results are given in table 2. For convenience, the first of the duplicate lots is designated as A, the second B.

The mean As_2O_3 content of the A samples was 0.00950 grain per pound, and of the B samples 0.00938 grain per pound. The mean of

the average of the A and B samples was 0.00944 grain, and the average deviation was 0.00070 grain, or 7.4 percent of the mean. For lead, the mean of the A samples was 0.02255 grain per pound, and for the B samples 0.02235 grain, while the mean of the averages of the A and B samples was 0.02246 grain per pound. The average deviation was 0.00152 grain of lead per pound, or 6.8 percent of the mean.

TABLE 2.—*Arsenic and lead (grain per pound)* [1] *found in duplicate samples of apples*

Sample	Arsenic as As_2O_3				Lead			
	A	B	Mean	Deviation	A	B	Mean	Deviation
B2	0.001	0.001	0.0010	0.0000	0.003	0.005	0.0040	0.0010
B17	.006	.007	.0065	.0005	.016	.018	.0170	.0010
B20	.016	.016	.0160	.0000	.028	.028	.0280	.0000
B21	.010	.011	.0105	.0005	.022	.026	.0240	.0020
B23	.001	.001	.0010	.0000	.003	.003	.0030	.0000
B25	.002	.002	.0020	.0000	.007	.008	.0075	.0005
B26	.012	.009	.0105	.0015	.028	.024	.0260	.0020
B27	.002	.002	.0020	.0000	.005	.013	.0090	.0040
B29	.008	.006	.0070	.0010	.022	.016	.0190	.0030
B30	.012	.010	.0110	.0010	.029	.027	.0280	.0010
B33	.018	.018	.0180	.0000	.033	.034	.0335	.0005
B34	.010	.012	.0110	.0010	.026	.026	.0260	.0000
B40	.005	.005	.0050	.0000	.013	.010	.0115	.0015
B46	.032	.032	.0320	.0000	.067	.077	.0720	.0050
B47	.042	.042	.0420	.0000	.082	.080	.0810	.0010
B48	.015	.016	.0155	.0005	.035	.039	.0370	.0020
B49	.007	.006	.0065	.0005	.013	.010	.0115	.0015
B51	.026	.024	.0250	.0010	.046	.055	.0505	.0045
B52	.007	.008	.0075	.0005	.021	.022	.0215	.0005
B59	.002	.002	.0020	.0000	.006	.008	.0070	.0010
B139	.006	.005	.0055	.0005	.018	.015	.0165	.0015
B146	.006	.006	.0060	.0000	.013	.016	.0145	.0015
B148	.011	.013	.0120	.0010	.032	.035	.0335	.0015
B149	.009	.011	.0100	.0010	.022	.020	.0210	.0010
B150	.012	.012	.0120	.0000	.035	.026	.0305	.0045
B152	.009	.008	.0085	.0005	.037	.025	.0310	.0060
B155	.011	.011	.0110	.0000	.040	.038	.0390	.0010
B224	.003	.002	.0025	.0005	.006	.004	.0050	.0010
B228	.001	.001	.0010	.0000	.003	.002	.0025	.0005
B230	.001	.002	.0015	.0005	.003	.005	.0040	.0010
B231	.002	.003	.0025	.0005	.005	.009	.0070	.0020
B232	.005	.005	.0050	.0000	.012	.007	.0095	.0025
B233	.005	.005	.0050	.0000	.012	.015	.0135	.0015
B234	.003	.002	.0025	.0005	.006	.004	.0050	.0010
B238	.011	.012	.0115	.0005	.028	.032	.0300	.0020
B239	.007	.004	.0055	.0015	.010	.008	.0090	.0010
A36	.005	.004	.0045	.0005	.011	.013	.0120	.0010
A37	.007	.006	.0065	.0005	.021	.019	.0200	.0010
A38	.005	.005	.0050	.0000	.010	.014	.0120	.0020
A39	.003	.003	.0030	.0000	.010	.008	.0090	.0010
A40	.004	.002	.0030	.0010	.004	.006	.0050	.0010
A41	.005	.007	.0060	.0010	.015	.011	.0130	.0020
A42	.012	.013	.0125	.0005	.038	.038	.0380	.0000
A43	.009	.010	.0095	.0005	.019	.021	.0200	.0010
A44	.011	.015	.0130	.0020	.034	.030	.0320	.0020
A45	.015	.017	.0160	.0010	.044	.048	.0460	.0020
A46	.007	.008	.0075	.0005	.018	.021	.0195	.0015
A47	.004	.004	.0040	.0000	.010	.010	.0100	.0000
A48	.007	.008	.0075	.0005	.019	.016	.0175	.0015
A49	.015	.010	.0125	.0025	.026	.026	.0260	.0000
A50	.006	.010	.0080	.0020	.032	.033	.0325	.0005
A57	.006	.007	.0065	.0005	.016	.020	.0180	.0020
A58	.004	.008	.0060	.0020	.014	.016	.0150	.0010
A59	.014	.009	.0115	.0025	.030	.026	.0280	.0020
A60	.009	.008	.0085	.0005	.021	.022	.0215	.0005
A61	.010	.009	.0095	.0005	.029	.032	.0305	.0015
A62	.007	.007	.0070	.0000	.018	.019	.0185	.0005
A63	.013	.012	.0125	.0005	.032	.036	.0340	.0020
A65	.010	.008	.0090	.0010	.025	.022	.0235	.0015
A66	.011	.011	.0110	.0000	.017	.019	.0180	.0010
A67	.011	.012	.0115	.0005	.031	.027	.0290	.0020
A81	.011	.009	.0100	.0010	.028	.025	.0265	.0015
A223	.014	.012	.0130	.0010	.029	.025	.0270	.0020

[1] Duplicate lots A and B.

TABLE 2.—*Arsenic and lead (grain per pound) found in duplicate samples of apples*—Continued

Sample	Arsenic as As_2O_3				Lead			
	A	B	Mean	Deviation	A	B	Mean	Deviation
A224	0.012	0.010	0.0110	0.0010	0.020	0.019	0.0195	0.0005
A225	.008	.009	.0085	.0005	.019	.020	.0195	.0005
A226	.019	.014	.0165	.0025	.025	.015	.0200	.0050
A227	.018	.012	.0150	.0030	.025	.021	.0230	.0020
A228	.012	.017	.0145	.0025	.020	.022	.0210	.0010
A233	.007	.009	.0080	.0010	.022	.017	.0195	.0025
A261	.020	.020	.0200	.0000	.032	.029	.0305	.0015
A304	.009	.010	.0095	.0005	.022	.026	.0240	.0020
A305	.005	.004	.0045	.0005	.014	.014	.0140	.0000
A306	.009	.009	.0090	.0000	.016	.014	.0150	.0010
A307	.004	.003	.0035	.0005	.012	.012	.0120	.0000
A312	.004	.004	.0040	.0000	.012	.014	.0130	.0010
A313	.002	.004	.0030	.0010	.008	.010	.0090	.0010
A316	.006	.004	.0050	.0010	.012	.010	.0110	.0010
A317	.003	.004	.0035	.0005	.011	.010	.0105	.0005
A318	.040	.035	.0375	.0025	.100	.090	.0950	.0050
A319	.024	.026	.0250	.0010	.075	.078	.0765	.0015
A322	.003	.006	.0045	.0015	.008	.012	.0100	.0020
A323	.003	.003	.0030	.0000	.009	.007	.0080	.0010
Average	.00950	.00938	.00944	.00070	.02255	.02235	.02246	.00152

The ratio Pb/As_2O_3 in the mean values is 2.37/1, and the ratio average deviation Pb/average deviation As_2O_3 is 2.15/1. These ratios, as well as the percentage values given in the previous paragraph, indicate that the error of the lead determination is not so great as that of the arsenic determination. Apparently the error of sampling affects the accuracy of the determination of these elements to much the same degree, and the increased accuracy of the lead determination reflects the greater accuracy of the chemical part of the procedure, mentioned previously. It is to be noted that the mean values for both arsenic and lead are close to the amounts permitted by law, and therefore the estimations of accuracy should be particularly applicable to samples falling in this range.

SUMMARY AND CONCLUSIONS

The errors incident to the determination of lead and arsenic as spray residues on apples are due to two sources: Errors of sampling and errors in the chemical determination of the elements. The latter may be further divided into errors of technique and errors of standardization.

When the chemical determination of lead by the photoelectric method is considered separately, the probable error was ± 0.0028 mg, when samples each containing 0.4000 mg of lead were analyzed. For the entire procedure, including both the chemical determination and the sampling, the mean amount of As_2O_3 present was 0.00944 grain per pound of fruit, with an average difference between duplicates of 0.00140 grain per pound. For lead the mean values were 0.02246 grain per pound of fruit, with an average difference between duplicates of 0.00304 grain per pound. These are the mean values of 164 apple samples analyzed in duplicate.

The lead determination was slightly more accurate than the Gutzeit arsenic estimation, even when the errors due to sampling were

considered. The average deviation of individual arsenic determinations from the mean of duplicate determinations was 7.4 percent of the mean; for lead it was 6.8 percent of the mean. These data are based on the analysis of 164 samples.

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